PHYSICO-CHEMICAL STUDIES OF BIOCHEMICAL/BIOLOGICAL SYSTEMS

ABSTRACT
THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

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IN

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By

SHEETAL SHARMA

DEPARTMENT OF CHEMISTRY
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ABSTRACT

Measurements of density, ultrasonic velocity and viscosity of acidic amino acids in the presence of salt solutions (1M, 2M CH_3COONa and 1M CH_3COOK) were made for investigating the intermolecular interactions. Aspartic and Glutamic acids have been chosen to observe the effect of CH_2 group on the volumetric and thermodynamic properties. The activity of egg-white Lysozyme was measured in the presence of carbohydrate additives (Glucose and Maltose) and urea in the reaction medium by accurate quantitative measurements of such properties as partial molal volume, adiabatic compressibility, B-coefficient, free energy of activation etc. The interaction of protein-water molecules in aqueous sugar and urea solutions and the temperature dependence of these interactions play very important role in understanding the thermodynamic processes in living cells.

Ultrasonic velocity data and the derived parameters such as adiabatic compressibility (β_s), change in adiabatic compressibility ($\Delta\beta_s$), and apparent molal adiabatic compressibility provide a basis for understanding the type and the extent of intermolecular interactions, such as weak or strong or no interaction at all. The decrease in compressibility with increase in the thermal breaking of solvent components, which in turn results in greater attractive forces among the molecules of a solution. Decrease in the β_s values with increase in composition is due to greater attractive forces among the molecules of a liquid.

Partial and transfer molal volume of acidic amino acids in the temperature range: 298.15-323.15K has been studied in different salt solutions. CH_2 group exerts an independent influence on the characteristics of adjacent water molecules, thus causing an increase in the partial molal volume. In Maltose, two glucose units are joined by α–1, 4 glycosidic linkage so the values of apparent molal volume of Maltose + Lysozyme + water systems are nearly twice in comparison to Glucose +Lysozyme +water system. Hydrophilic- hydrophilic interactions between the -OH groups of monosaccharide and the hydrophilic R group present on the exterior of Lysozyme structure are responsible for the positive values of transfer volume while nonpolar-hydrophillic interactions between the nonpolar Urea and hydrophilic R group of Lysozyme present on the exterior are responsible for negative values of transfer volumes.

Viscosity (η) measurement provides valuable information about the size and shape of the molecules. The values of η have been calculated for all the systems under investigation and that they are found to increase with concentration and decrease with increase in temperature except in the case of Urea in Lysozyme solution. In the case of Urea in Lysozme solution, viscosity first decreases from 0.02 mol/kg to 0.06 mol/kg and then it gradually increases. B-coefficient of Jones-Dole equation has also been evaluated by using the viscosity data. Change in enthalpy (ΔH^*), entropy (ΔS^*) and free energy of activation (ΔG^*) have been evaluated from viscosity data. ΔG^* has been found to increase linearly with temperature.

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Dated: 8.11.06

Certificate

This is to certify that the work presented in this thesis entitled **"Physico-Chemical Studies of Biochemical/Biological Systems"** is original work carried out by **Ms. Sheetal Sharma**, under my supervision and is suitable for submission for the award of Ph.D. degree in Chemistry of this University.

Dr. (Mrs.) Saeeda Naqvi

In the

Everlasting Memory of

My

Ever Inspiring

Grandfather

(Late) Sh. S.P. Sharma

Head of English Department

N.R.E.C. (P.G.) College, Khurja

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Sheetal Sharma

Sheetal Sharma

CONTENTS

General Introduction

Proteins play key role in virtually all biological processes. Nearly all catalysts in biological systems are proteins called enzymes. Proteins mediate a wide range of other functions, such as transport and storage, coordinated motions, mechanical support, immune protection, excitability, integration of metabolism and control of growth and differentiation [1].

The basic structural units of proteins are amino acids. All proteins in all species from bacteria to humans are constructed from the same set of 20 amino acids. The side chains of these building blocks differ in size, shape, charge, hydrogen-bonding capacity and chemical reactivity. The overall behaviour of proteins mainly depends on the type and order of arrangement of amino acids. Proteins are stabilized by many reinforcing hydrogen bonds and van der Walls interactions as well as by hydrophobic interactions. Proteins are a unique class of macromolecules in being able to specifically recognize and interact with highly diverse molecules [1].

Lysozyme is an enzyme in egg white and human tears that catalyzes the hydrolytic cleavage of complex polysaccharides in the protective cell walls of some families of bacteria. Lysozyme is so named because it can lyse, or dissolve bacterial cell walls and thus serve as a bacterial agent. Like myoglobin and cytochrome C, lysozyme has a compactly folded conformation and has most of its hydrophobic R groups inside the globular structure, shielded from water and its hydrophilic R groups outside, facing the aqueous medium [2,3]. This highly stable protein is cross-linked by four disulfide bridges. In 1965 David Phillips and his colleagues determined the three dimensional structure of lysozyme. Their high-resolution electron density map

shows that lysozyme is a compact molecule roughly ellipsoidal in shape, with dimensions 45×30×30Å. The folding of this protein is complex. About 40 percent of its 129 amino acid residues are in α-helical segments, much less than in myoglobin and hemoglobin. In a number of regions, the polypeptide chain is an extended β-sheet conformation. The interior of lysozyme, like that of myoglobin and hemoglobin, is almost entirely non-polar. Hydrophobic interactions play an important role in the folding of lysozyme [1].

Its structure, dynamics and hydration have recently been studied extensively by a wide range of experimental techniques including 1H, 13C and 15N-NMR spectroscopy [3-8], dielectric spectroscopy [9-11], Fourier transform infrared spectroscopy [12-13] and X-ray crystallography [14]. Some sophisticated theoretical methods have also been applied to those problems in the literature [15-17]. In a solution, the lysozyme molecules are surrounded by water. It participates in stabilizing the protein structure and stimulating the activity of active site. Comparison of solution NMR parameters with those predicted by the crystal structures of the protein has shown the very close similarity of the structure of lysozyme in solution and in crystals. Although recent X-ray diffraction studies of dehydrated lysozyme crystals have revealed numerous small displacements in the positions of individual atoms, the overall conformation does not differ greatly from that of the fully hydrated protein [18].

The study of interaction with water of different hydrophobic and hydrophilic groups has become a topic of general interest. Water itself is now believed to possess a degree of structural organization, sometimes loosely called "water structure". In terms of recent theories

[19-21], it is appropriate to consider bulk water as consisting of flickering clusters of hydrogen-bonded molecules in which the cooperative nature of cluster-formation and relaxation is related to the partially covalent character attributed to the hydrogen bond. While considering the interaction of a given group with water, it is necessary to ask in what way such interactions may be influenced by a change of "water structure" itself. Such a change would be expected to be produced by the presence of a solute whether polar or non polar. The presence of an ion, in particular, should result in significant contributions towards restructuring the medium in its vicinity. Franck and Wen [21] have envisaged three zones over which the action of an ion should be exercised. A narrow region A, in which the nearest neighbouring water molecules are always essentially immobilized by direct ion-dipole interaction. Beyond this narrow layer, could exist another region B, in which water could be less structured (ice-like), i.e., more random in organization than "normal", and a third region C which contains the structurally "normal" water. The net effect of the ion on water structure presumably results from the superposition of these competing effects in region B. This general picture has provided a way of accounting for experimental results in a variety of areas including entropy, heat capacity, thermal conductivity and dielectric relaxation of solutions. Frank and Evans [22] have made entropy data the basis of assigning an orderly gradation of net structure-altering influence to a number of ions.

A different case of interference with water structure should occur with non-polar solutes or with non-polar groups in a solute molecule. This is a structure-making influence, again suggested by Frank and

Evans [22] from entropy data. In the picture suggested by these authors, the statistical degree of "ice-likeness" of the sample is proportional to the average size and to the average half-life of the clusters. "A cluster comes into existence when a volume element of suitable size and shape suffers a negative energy fluctuation of such a magnitude as to outweigh the disruptive influences of the boundaries and will dissolve when influences such as torque and displacements succeed in transmitting into the cluster the necessary energy of melting". A non-polar solute particle, due to the feebleness of the electrostatic interactions into which it can enter, should be relatively incapable of transmitting such perturbations and thus favour the formation of "ice-likeness" in its vicinity. This ice-likeness is assumed to melt with a rise in temperature at a proportional rate not smaller than that characterizes "normal ice-likeness", the contrary is true for a solute, which impairs an extra randomness, i.e., which acts as a structure- breaker.

Water can be conceptualized as a mixture of two rapidly inter-converting species: a less dense, more structured species, and a more dense, less structured species [23-28]. Although this two-State mixture approximation has been criticized for its simplicity [29,30]. It has proven useful in understanding the volumetric properties of solutions [27]. According to this model and Le Chatlier's principle, increasing either the temperature or the pressure increases the fraction of the more dense less structured species at the expense of the other species [24,25-31]. Because it takes heat to break the structure, a decrease in structure will decrease the heat capacity of pure water, making Cp inversely proportional to P at constant T[$(\partial Cp/\partial p)T < 0$].

An analogous idea applies to a solute's effect on water structure. One model of solute hydration states that the differences between bulk water and hydration water arise from a varying ratio between the more dense and less dense water species [27]. A structure-making solute increases the fraction of the less dense species at the expense of the more dense species in the solute's hydration sphere. A 'structure breaking solute has the opposite effect.

The study of carbohydrate/saccharides has become a subject of increasing interest because of the multidimensional physical, biochemical, and industrially useful properties of these compounds [32-38]. These are important chemicals in life processes, as these constitute a part of glycoproteins, glycolipids, and other biomolecules. Because of their conformational flexibility, saccharides play significant role in (bio) molecular recognition. However, the understanding of the relationship between saccharide structures and their biological function is still far behind that of proteins and nucleic acids [39-42]. Saccharides have received considerable attention for their ability to protect biological macromolecules [43,41]. Sugars and polyols are well known stabilizing agents for the native state of proteins/enzymes [44,45] because of their ability to enhance the structure of water. Various thermodynamic [38,46-50] and spectroscopic studies [50,51] have shown that the hydration of saccharides depends upon the number of hydroxyl groups [52], the potential hydrogen-bonding sites and relative position of the next nearest-neighbour hydroxyl groups within the carbohydrate molecule [48,53].

Stokes and Robinson [54] interpreted the concentration dependences of activity coefficient of D(-) glucose and sucrose in terms

of the semi-ideal solution theory. This idea that the solute-solvent interactions govern the concentration dependences of thermodynamic quantities seems to be fundamentally correct, since Raman [55] and NMR relaxation [56] studies of aqueous solutions of mono and disaccharides suggested an absence of solute-solute interactions even at relatively high concentrations.

Kabayama and Patterson [57] postulated that not only the number of hydroxyl groups but also the stereochemical orientation of hydroxyl groups, i.e., axial and equatorial, plays an important role on the hydration of saccharides. In other words, the spacing of oxygen atoms of equatorial OH groups of monosaccharides in chair conformation nearly fits the spacing of oxygen atoms of ice like structure of water. This hydration model for saccharides is called "specific hydration model".

Proteins are stabilized by a combination of hydrogen bonding interactions, electrostatic interaction and hydrophobic interactions. In aqueous solutions of proteins there is a cooperative hydrogen-bonding structure [58] in which water competes as both donor and acceptor with the backbone and side chain groups of the protein. When sugar is added to the protein solution, the OH group of sugar may also compete for hydrogen bonding [58].

The denaturation process can be achieved by any one of the following methods: increasing temperature, changing pH, using denaturants (i.e. urea, guanidine hydrochloride), inorganic salts (i.e. lithium bromide, potassium thiocynate, sodium iodide), organic solvents (i.e. formamide, dimethylformamide, dichloro-and tricholoracetic acids and their salts), detergents (i.e. sodium dodecyl

sulphate), high pressure and ultrasonic homogenization. In the present study we have made an attempt to study the effect of denaturant urea.

The temperatures at which various proteins unfold vary enormously. The denaturation of certain proteins in the presence of denaturants like urea and guanidine hydrochloride is reduced in the presence of sugars or polyhydroxy alcohols. However, the mechanism is not clearly understood by which this denaturating effect on protein structure is induced either by direct binding of urea and guanidine hydrochloride with protein molecules /polyhydroxy compounds or through the alteration of water structure.

Protein denaturation has been defined in several ways, for example as a change in solubility or by simultaneous change in chemical, physical, and biological properties under some standard reference set of conditions. These changes in physical, and to a lesser extent chemical properties are manifestations of configurational changes taking place in the polypeptide chains. The denaturation process presumably involves an unfolding or at least an alteration in the nature of the folded structure. Most denaturation changes consist of changes in secondary bonds: ion-dipole, hydrogen and van der Waals, and in rotational position about single bonds, which are controlled by the secondary bond structure. The term denaturation denotes the response of the native protein to heat, acid, alkali, and variety of other chemical and physical agents, which cause marked changes in the protein structure. Denaturation means a class of reactions, which lead to changes in the structure of the macromolecule with no change in molecular weight. The knowledge of solute-solvent and solute-solute

interactions in various solvents is thus, prerequisite to understanding the process of denaturation.

Several factors contribute to tertiary structure of a protein and changes in any of them could affect its structural integrity and biological activity. When a protein is heated to an extreme temperature, the balance of non-covalent interactions maintaining the native structure is disrupted and the protein unfolds partially. This process disturbs the active site of the enzyme [59]. Similar behaviour is observed with the denaturating agents like urea and sodium dodecyl sulphate (SDS). The characterization of stable intermediate states would be of great help for the proper understanding of the overall process of protein folding [60]. X-ray crystallography data have revealed that some packing defects or cavities in a protein molecule [61] cause fluctuations, which are related to the structural characterization and functional properties of proteins. However, a complete understanding to ascertain the role of fluctuations in protein functions and biochemical phenomenon require further investigations on the magnitude of the flexibility of protein molecules in various solvents and at different pH values. Since the fluctuation in volume is directly related to the compressibility [62], the flexibility of protein should be reflected in the compressibility and the latter is primarily related to its thermal stability.

Urea, the first synthetic organic compound, occupies a unique position, not only in its wide commercial applications but also in its physiological importance. Incorporating in its structure the most important functional groups involved in life processes, viz. >C=0 (carbonyl) and -NH2 (amino), with C-N bonds acquiring a partial

double bond character in its canonical forms, it forms the smallest molecule applicable for study of most complex reactions of the living cell. Significant research effort has dealt with understanding urea relatively in both catalytic and non-catalytic media [63-73]. Nonetheless, there still is uncertainty regarding the interpretation and nature of the aqueous phase reactivity of urea, for which unimolecular elimination and biomolecular elimination/hydrolysis mechanisms have been described [70,71]. Moreover, urea is unusually stable because of its resonance stabilization (estimated to be 30-40 kcal/mol) [74], which decreases the electrophilicity of the carbonyl carbon. These mechanisms are related, respectively, to intramolecular and intermolecular proton-transfer reactions involving both amino groups or the amino and the carbonyl groups. The intermolecular mechanism proceeds with the assistance of a water molecule and can follow elimination or hydrolytic pathways. The theoretical treatment of the urea-water mixtures has been carried out by Frank and Franks [75]. According to them urea acts mainly to disrupt hydrogen bonding among water molecules by dissolving exclusively within the dense (less structured) component of liquid water and with inducing alternate types of long-range structure. The structure breaking influence of urea on water was supported by NMR results of Finner et al [76]. The structure breaking/making effects of urea on water may also be interpreted in terms of the pair-wise interaction coefficients of the virial expansions of the excess Gibbs free energy, enthalpy and entropy. On the basis of the sign and magnitude of pair-wise enthalpic, Hxx, entropic, Sxx and Gibbs free energy, Gxx, coefficients, urea is considered to be a hydrophilic structure breaker solute [77]. Its presence as a co-solvent lowers the degree of structure of the medium with respect to the reference state, namely pure water,

because its polar geometry is not compatible with the tetrahedral arrangement of water molecules. Thus, the presence of urea enhances the entropy and enthalpy of the bulk water. When solvated solute molecules interact in the presence of urea, the change in enthalpy and entropy could be more marked compared with that occurring in pure water. The presence of urea also modifies the solvation co-sphere of the solute; therefore, it is generally accepted that urea does not appreciably interact with either hydrophobic or hydrophilic molecules or groups, and acts mainly to disrupt hydrogen bonding among water molecules in aqueous medium. Mathieson and Conway [78] reported that thermodynamic and transport properties of aqueous urea solutions with added electrolytes or non-electrolytes have been interpreted in terms of non-specific interaction of urea with solute. The values of the thermodynamic functions were found to be consistent with the hypothesis that urea solution is similar to water but less structured. Aqueous solutions of urea and its derivatives are an important mixed solvent with wide range of scope and purpose [79]. The structure of urea water mixtures is also of great importance in understanding protein denaturation.

Acidic amino acids (with an extra-COOH on the side chain) are negatively charged at the pH of the cell and thus hydrophilic. Aspartic acid and glutamic acid play important roles as general acids in enzymes active centers as well as in maintaining the solubility and ionic character of proteins. Aspartic acid is alanine with one of the β-hydrogens replaced by a carboxylic acid group. Glutamic acid has an additional methylene group in its side chain than does aspartic acid. The use of the acidic amino acids shows the effect of CH2 group on the

volumetric and thermodynamic properties. The partial molal volume values vary linearly with the number of carbon atoms in their alkyl chains at given temperature. A linear correlation has been reported for the homologous series of ω-amino acids in aqueous potassium thiocyanate solution [80].

The physical properties of dilute aqueous solution of non-electrolyte depend on whether the solute is a water structure breaker or maker. The influence of small quantities of amino acids on the hydrogen bonded structure of water in the solution of water rich water-salt mixture is quite different from that in the absence of amino acids.

There are extensive volumetric property studies of aqueous amino systems, but few of amino acids in mixed aqueous solvents [81-83, 80]. A small change in water structure can greatly inhibit the physiological reactions in cells or tissues, which are made up of biological macromolecules. The change in water structure can be brought about by the presence of electrolytes. Sometimes these changes are helpful in controlling undesired physiological reactions [84-89] occurring in living organisms.

Salt solutions have large effects on the structure and the properties of proteins including their solubilization, denaturation, dissociation into sub-units and the activity of enzymes [90-92]. The complex conformational and configurational factors affecting the structures of proteins in various solvents make the direct interpretation on proteins very difficult. Therefore, investigations of the behaviour of model compounds of proteins like amino acids and peptides are of importance.

There have been many studies on the amino acid-water-salt systems. As far as the volumetric properties are concerned, the infinite dilution apparent molar volumes for some amino acids have been determined in aqueous calcium chloride [93], alkali metal halide (LiCl, NaCl, KCl, CsCl, KBr, KI] [94-97], potassium thiocyanate [80,98], ammonium chloride [99] and guanidine hydrochloride [82,100], solutions. Sodium acetate (Na+, CH3COO-) is known to influence the dissociations of proteins in solution [101] and cause a salting out of polar non-electrolytes [102]. Acetate ion has a hydrophobic methyl group and a carboxylate ion residue; its effect on amino acids should be different from the simple anions. Therefore, it is interesting to investigate the behaviour of model compounds of proteins in aqueous sodium acetate solutions and in aqueous potassium acetate solutions at different temperatures. Sodium and potassium salts have been chosen for this part of the study to observe the relative effects of the change of cations of the electrolyte.

The denaturation and reactions of proteins under the high static pressure or ultracentrifugal force have been a matter of concern for many investigators [103-106]. In such works, the compressibility of native protein in solution has been an indispensable quantity to analyze, in detail, the obtained results. The protein compressibility in solution has been thus far estimated by following two methods. One is the measurement of the partial specific volume of protein in solution as a function of pressure by the direct densimetric method [107,108]. This method gives isothermal compressibility data. Another method uses sound velocity measurement with an ultrasonic interferometer [109,110]. The compressibility obtained by this technique is an

adiabatic. An important result in these compressibility studies is that globular proteins have a positive compressibility while the constituent amino acids have negative ones due to the hydration effect. This result suggests that the compressibility of the protein interior is very large. At present, however, it is difficult to understand the compressibility of proteins on a molecular level because few compressibility data [107-110] have been reported, probably due to technical difficulties. The partial molar volume of a protein is known to result from three contributions [111]-

(i) The constituent atomic volume

(ii) The void volume because of imperfect atomic packing and

(iii) The volume change due to solvation.

Since the constituent atomic volume should be approximated as incompressible, compressibility data of globular proteins in water will produce useful information on the internal structure and the hydration structure of protein, which are still obscure. Furthermore, such compressibility data should present important information to the understanding of the mechanisms of pressure induced denaturation or reactions of proteins. Ultrasonic velocity and its derived parameters have been extensively used to study the molecular interactions in solutions.

Ultrasonic velocity values along with density data can be employed for the computation of various thermodynamic parameters, namely adiabatic compressibility, and compressibility lowering etc., which are helpful in knowing the nature of various interactions occurring in a solution.

The solute-solvent interactions have been studied in the light of thermodynamic property viz. the compressibility lowering $\Delta\beta$. Thus the study of $\Delta\beta$ will ultimately lead to a better understanding of the influence of molecular configuration on interactions. Compressibility lowering was evaluated as the difference in the compressibilities of solvent and solutions [112].

The effects of hydrogen bonding [113-115], electrostriction [116-118], ionization [118-124], hydrophobic interaction [56], and zwitterions formation [125] etc. on the partial molal volumes of the solutes can be estimated. Apparent molal volumes of several α-amino acids were determined by Millero et al [126], who calculated the number of water molecules bonded to the charged centers of the α-amino acids. Recently, many authors have investigated the aqueous solution volumetric properties of some protein constituents, viz., the amino acids and peptides [125,115,126-128] for different reasons. They also estimated various group contributions towards limiting partial molal volumes.

Apparent molal volume and apparent molal compressibility are very sensitive to interactions between solute and solvent and to changes induced in the solvent by the solute.

The transport properties in solutions are studied by measuring the viscosity of solutions. The viscosity measurement of macromolecules provides information regarding the shape and size of these molecules [129]. The viscosities of electrolyte solutions were considered by Falkenhagen and Dole [129] in terms of the interionic interactions in the adjacent layers of an electrolyte solution. They proposed that the electrical forces between the ions in a solution tend to

establish and maintain a preferred rearrangement and thus to stiffen the solution, i.e., to increase its viscosity.

Among the physicochemical properties, which are utilized for the product designing and optimization, viscosity (η) is a very important property.

The viscosity data have also been interpreted by several workers in terms of Jones-Dole equation [130-136]. They have introduced the viscosity coefficient β for the dipolar ions, particularly amino acids. It is argued that the sign of the temperature dependence of the β-coefficient provides a more satisfactory information about the structure making or structure breaking ability of the solutes on the solvent than the sign of the β-coefficient.

Therefore, viscosity measurements on protein are useful in indicating the changes in the molecular configuration due to denaturation. When a rigid molecule of low axial ratio is unfolded into flexible chains, there is an observed increase in viscosity. Viscosity measurements of solutions that convert compact proteins to the random coil configuration can be related to the size of the polypeptide chain. This can also tell us whether different denaturants acting on a protein produce molecules differing in shape or properties.

Most proteins unfold at elevated temperatures, and some unfold at very low temperatures. Many proteins unfold at temperatures only a few degrees higher than those at which they function. Others are stable to much higher temperatures such as the gluten proteins. The driving force for denaturation is the increase in entropy that accompanies the transition of a single conformation into an ensemble of random ones. With increasing temperature, the contribution of this entropy increases

and becomes more significant, and at some temperature it overcomes the energy effect (the protein is heat denatured). It is interesting to consider possible intermediate structures. The early unlocking of the tertiary structure deletes a large number of the bonds holding the structure together but increases the randomness only insignificantly. The later stages of denaturation lead to larger increases in entropy.

In the present work, an attempt has been made to probe the behaviour of acidic amino acids in the presence of salt solutions (1M and 2M CH3COONa and 1M CH3COOK) as functions of temperature and concentration. In order to understand the effects of sugars and urea on protein-water system, an aqueous solution of lysozyme (0.15 mili-molal) was prepared and varying amount of sugars (D(-) glucose, maltose), or urea were added to this solution and various physical parameters have been calculated using densities, viscosities and ultrasonic velocities, which were measured as functions of concentration and temperature.

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Experimental

Material and Sample Preparation

The two amino acids, aspartic acid and glutamic acid of highest purity for biochemistry (chromatographically homogeneous) were obtained from Sisco Research Laboratories Pvt. Ltd. Mumbai, India and were used without further purification. However, before use they were dried over P_2O_5 in a vacuum desiccator. Analytical reagent grade anhydrous sodium and potassium acetates and urea crystal extra pure were obtained from Merck Limited Worli, Mumbai. Sodium and potassium acetates were further purified by recrystallising from double distilled water. After recrystallisation sodium and potassium acetates were dried under vacuum at room temperature. Lysozyme obtained from SIGMA-ALDRICH CHEMIE Gmbh Steimhein, Germany, was used for sample preparation. Sugars viz. D-glucose and maltose were obtained from Qualigans fine chemicals (a division of Glaxo Smith Kline Pharmaceuticals Limited, Mumbai). The solutions were prepared by weight with laboratory double distilled water and all weights were corrected to value in vacuo (Leader balance Works, Varanasi, U.P).

The densities, viscosities and ultrasonic velocities were determined for the following solutions:

1. Aspartic acid and glutamic acid in CH_3COONa solutions of different concentrations (1M and 2M).
2. Aspartic acid and glutamic acid in 1M CH_3COOK solutions.
3. D-glucose in aqueous lysozyme solution.
4. Maltose in aqueous lysozyme solution
5. Urea in aqueous lysozyme solution.

Temperature Control

For the measurements of density and viscosity, a thermostated paraffin bath was used to maintain a uniform temperature. The paraffin bath was of about 5 litres' capacity in which an immersion heater (1.0 KW), an electric stirrer (Remi made), a check thermometer, a contact thermometer were immersed. A relay [Jumo type NT 15.00, 220V ≈ 6A (GDR)] was used to control the variation in temperature. The thermal stability was found to be within $\pm$ 0.1°C.

Calibration of pyknometer

Pyknometer is an apparatus used for measuring the density of a liquid. It consists of a small bulb with a flat bottom (of about 9 ml capacity) and a graduated stem for measuring the density of the experimental liquid. It is etched with very fine marks. Each mark on the stem of the pyknometer was calibrated using double distilled water. The clean and dried pyknometer was weighed and filled with double distilled water. Filled pyknometer was weighed again. The mass of the distilled water was determined by the difference in these two masses. Then the pyknometer was immersed in the paraffin bath maintained at the required temperature, and volume changes were recorded as a function of temperature, and thus each mark of the stem was calibrated. The density of distilled water at different temperatures required for calibration was given by the standard equation:

$$d = A_0 + A_1 t + A_2 t^2 + A_3 t^3$$

where A_0, A_1, A_2 & A_3 are constants and the values of A_0, A_1, A_2 and A_3 are 1.0004238, $-3.6067599 \times 10^{-6}$, $-5.6632867 \times 10^{-6}$ and 1.5613054×10^{-8}, respectively, while t is the temperature in ^{0}C.

From the known values of mass and density of water, the volume corresponding to each mark of the pyknometer was determined. Reproducibility of calibration was checked by repeating the above procedure with different weights of distilled water. Using the known values of mass and volume, the densities at the required temperature were determined. The values of the observed densities were compared with those of the reported ones. It was found that the accuracy of the measurement was within ± 0.1% accuracy.

Calibration of Viscometer

Cannon-Ubbelohde Viscometer [1] was used for the determination of viscosities of solutions.

The viscometer consists of three parallel arms viz., receiving, measuring and auxiliary, for forming the suspended level arrangement in a triangular fashion. The measuring arm has a fine capillary tube with two bulbs A and B. It forms a 'U' with the receiving arm. The measuring arm is etched with two marks (a & b), one above the bulb B and the other below the bulb B. The two fudicial marks 'a' and 'b' were used for recording the time of fall of the test solution. The viscometer was designed in a manner so that (1) the center of gravity of the three bulbs was aligned vertically to reduce the effect of acceleration due to gravity and (2) the resulting efflux time for water was set close to 80 seconds at room temperature (depending upon the dimensions of

viscometer). In order to minimize the experimental errors, capillary effects of the two liquid surfaces were neutralized by each other, so that the surface tension correction for the apparatus was negligible and the transport of material was carried out freely under the weight of the total volume of the test liquid.

The calibration of viscometer was done by using the distilled water. A sufficient amount of distilled water was filled into the bulb A to avoid any air bubble being introduced into the capillary arm while the bulb B was filled. Now the viscometer was clamped in a thermostat keeping the measuring arm perfectly vertical. The viscometer was allowed to stand in the thermostat for half an hour to minimize thermal fluctuations.

Then the distilled water was sucked into the measuring bulb with the help of vacuum pump. The time of fall of the distilled water from the upper mark 'a' to lower mark 'b' was recorded several times and the mean of very close readings was determined at each required temperature. A stop-watch (accuracy: 0.1 second) was used for measuring time.

Viscosities (η) were calculated using Poiseuille's equation:

$$\eta = \frac{\pi h \rho g r^4 t}{8LV}$$

where h = height of the liquid column in the viscometer

ρ = density of the liquid

g = acceleration due to gravity

r = radius of the capillary of the viscometer

L = length of the capillaries

t = time of fall of the test liquid of volume V to fall through capillary.

The expression can be written in this way also

$$\eta = \rho\beta t$$

where $\beta = \frac{\pi hgr^4}{8LV}$.

β is a constant quantity and it is the characteristic of the viscometer. Its value has been calculated by making use of the reported values of viscosities of distilled water at several temperatures.

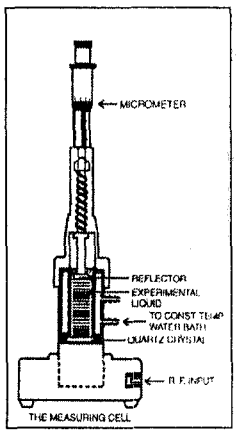
The accuracy of the calibrated viscometer was checked by measuring the viscosities of distilled water at various temperatures and then comparing the experimental value with the reported ones. Reproducibility was found to be within $\pm$ 0.2%.

Measurements

1. **Density:** A known amount of test sample was transferred to the calibrated pyknomter. The pyknometer was then immersed in the thermostated bath. The volume corresponding to each of the marks was recorded as a function of temperature.

2. **Viscosity:** The test solution was transferred to the viscometer. The viscometer was then placed in the thermostat and time of fall of the test solution was recorded.

Ultrasonic Velocity



Working Principle:

An ultrasonic interferometer is a simple and direct device to determine the ultrasonic velocity in liquids with a high degree of accuracy.

The principle used in measurement of velocity (v) is based on the accurate determination of the wavelength (λ) in the medium. Ultrasonic waves of known frequency (f) are produced by a quartz plate fixed at the bottom of the cell. The waves are reflected by a movable metallic plate kept parallel to the quartz plate. If the separation between these two plates is exactly a whole multiple of the ultrasound wavelength, standing waves are formed in the medium. The acoustic resonance gives rise to an electrical reaction on the generator, driving the quartz plate and the anode current of the generator becomes maximum.

If the distance is now increased or decreased and the variation is exactly one half wavelength ($\lambda/2$) or a multiple of it, anode current again becomes maximum. From the knowledge of wavelength (λ), the velocity (v) can be obtained by the relation:

Velocity = wavelength x frequency

$$v = \lambda \times f$$

Description

The ultrasonic interferometer consists of the following two parts:

i. The high frequency generator

ii. The measuring cell

The high frequency generator is designed to excite the quartz plate fixed at the bottom of the measuring cell at its

resonant frequency to generate ultrasonic waves in the experimental liquid in the "Measuring Cell". A microammeter to observe the changes in current and two controls for the purpose of sensitivity regulation and initial adjustment of micro ammeter are provided on the high frequency generator.

The Measuring Cell is a specially designed double walled cell for maintaining the temperature of the liquid constant during the experiment. A fine micrometer screw has been provided at the top, which can lower or raise the reflector plate in the cell through a known distance. It has a quartz plate fixed at its bottom.

Adjustment of Ultrasonic Interferometer

The instrument was adjusted in the following manner:

1. The Cell was inserted in the square-base socket and was clamped to it by a screw provided on one of its sides.
2. The curled cap of the cell was unscrewed and removed, then the test solution was filled in it and the cap was screwed.
3. Water was circulated through the two tubes in the double walled cell in order to maintain the desired temperature during the experiment.
4. The Cell was connected with a high frequency generator by a coaxial cable provided with the instrument.
5. The generator was given 15 seconds warming up time before recording readings.
6. The sudden rise or fall in temperature of the circulated liquid was avoided to prevent the thermal shock to the quartz crystal.

For the initial adjustment, two knobs are provided on the

high frequency generator, one is marked with "Adj" and the other with "Gain", the knob marked with "Adj" was used to adjust the position of the needle on the ammeter and the knob marked with "Gain" was used to increase the sensitivity of the instrument for greater deflection. The microammeter was used to record the maximum deflection by adjusting the micrometer screw.

Measurement

The Measuring cell is connected to the output terminal of the high frequency generator through a shielded cable; the cell is filled with the experimental liquid before switching on the generator. The ultrasonic waves move normal from the crystal till they are reflected back from the movable plate and the standing waves are formed in the liquid in between the reflector plate and the quartz crystal.

The micrometer is slowly moved till the anode current on high frequency generator shows a maximum. A number of maximum readings of anode current are passed on and their 'n' is counted. The total distance (d) thus moved by the micrometer gives the value of wavelength (λ) with the help of the following relation,

$d = n \times \lambda/2.$

Once the wavelength (λ) is known, the velocity (v) in the liquid can be calculated with the help of following relation:

$v = \lambda \times f$

Study With Variation in Temperature

If the variation in the velocity with temperature is to be studied, water at various desired constant temperatures is made to circulate through the double walled jacket of the cell. The

ripples are provided at the lower cylindrical portion of the cell for circulating water around the experiment liquid.

Ultrasonic velocity in solutions was measured by determining the wavelength of sound in these media using a multi-frequency ultrasonic interferometer model M-82 (Mittal Enterprises, India) working at 2MHz. The temperature of the solution was controlled by circulating water through the jacket of a double walled cell from a constant temperature controlled bath of thermal stability: $\pm$ 0.03K.

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Chapter-1

Volumetric and Adiabatic compressibility behaviour of acidic amino acids in salt solutions

Proteins can be denatured by heat, extremes of pH and by chemical agents like urea and guanidinium chloride, etc. At very low pH, the denaturation of protein is due to repulsion of positively charged residues. If large-size anions are introduced into the solution, the protein acquires the "A" state, also called the molten-globule state [1], which has been proposed as an important intermediate form, and could be a key step in understanding the protein folding problem.

The extent of solute hydration is modified by changes of temperature, pressure and the presence of co-solute. Most of the previous studies on amino acids have been restricted to water at a given temperature, but interesting results have been obtained when the studies have been extended to changes in temperature, pressure and use of a mixed aqueous solvent. It is useful to extend the study of amino acids to a mixed solvent system not only because mixed aqueous solvents are used extensively in chemistry and other fields to control factors such as solubility, reactivity and stability of systems but also because biological fluids are ultimately not pure water [2-5].

Salt solutions have large effects on the structure and properties of proteins, including solubility, denaturation, dissociation into sub-units, and the activity of enzymes [6]. The unusual increase in the stability of apoflavodoxin at neutral pH effected by salts is reported [7] to be likely a common property among highly acidic proteins. The stability and folding kinetics of wild-type and a mutant staphylococcal nuclease (SNase) at neutral pH are reported to be significantly perturbed by the

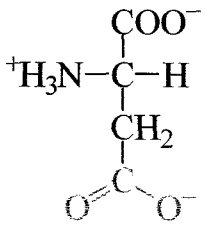
presence of salts [8]. The salt induced effects on the kinetics of folding have been attributed to the enhanced stability of transient folding intermediates.

Volumetric data on the constituents of proteins in the presence of salts is scarce. Therefore, in order to understand the finer details of the behaviour of proteins in aqueous salt solutions, the volumetric and compressibility properties of carboxylic amino acids (Aspartic acid and Glutamic acid) in 1M and 2M sodium acetate and 1M potassium acetate solutions at different temperatures are studied. Sodium and potassium salts have been chosen for this part of the isolate study to observe the relative effects of the change of cation of the electrolyte.

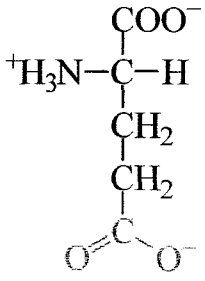
All the different types of proteins are initially synthesized as polymers of α-amino acids. At neutral pH, α,ω–amino carboxylic acids exist as zwitterions containing two oppositely charged carboxyl and amino groups separated by a non branched chain of CH_2 groups: NH_3^+-$(CH2)_n$-COO^- . There are obvious advantages for choosing α,ω amino acids as model systems for studying hydration effects of charged and hydrophobic atomic groups: (i) they do not contain other atomic groups, which might influence the hydration of the aliphatic and/or charged groups; (ii) the distance between the carboxyl and amino groups can be varied systematically by changing the number of aliphatic CH_2 groups in the molecule, thereby allowing one to study the interaction of the charged ends as a function of the distance between them; (iii) the presence of two charged groups at the termini of the hydrophobic

chain provides sufficient solubility of long homologues, a prerequisite for volumetric measurements [9].

An ionized group is very hydrophilic. Acidic amino acids (with an extra-COOH on the side chain) are negatively charged at the pH of the cell and thus hydrophilic. Aspartic and glutamic acids are the acidic amino acids. Aspartic acid contains a carboxylic acid group separated by a methylene carbon ($-CH_2-$) from the α-carbon (Fig. 1(a)). In glutamic acid, the carboxylic acid group is separated by two methylene ($-CH_2-CH_2-$) carbon atoms from the α-carbon (figure 1(b)). At physiological pH, side chain carboxylic acid groups are unprotonated and negatively charged [10,11].



Aspartic Acid
Fig. 1(a)



Glutamic Acid
Fig. 1(b)

The side chain-COO^- groups of Aspartic and glutamic acids have pK_a values around 4 so that they are virtually dissociated at pH 7.4; these provide the negatively charged acidic side groups of proteins.

The structure of proteins is influenced to a large extent by the charge state of ionizable groups on the side chains of several amino acids [12]. At pH below neutrality, the amino acids mainly involved in the ionic equilibria are glutamic acid (glu) and

aspartic acid (asp), with carboxylate pK of 4.33 (at 5^0C) and 4.01 (at 1^0C), respectively [13].

The volumetric and compressibility behaviour of a solute in solution can provide information concerning solute-solvent and solute-solute interactions. Ultrasonic velocity data as such does not provide significant information about the native and relative strength of various types of intermolecular/interionic interactions, but the derived parameters such as adiabatic compressibility (β_s), change in adiabatic compressibility ($\Delta\beta_s$), and apparent molal adiabatic compressibility provide a basis for understanding the type and the extent of intermolecular interactions, such as weak or strong or no interaction at all, and may throw some light quantitatively on the mechanism of intermolecular processes. A departure from linearity in the ultrasonic velocity versus composition in liquid mixtures is taken as an indication of the existence of interactions between different species [14,15].

Theory

Adiabatic compressibility, β_s, is calculated from the experimental value of sound velocity, U, and the density, ρ, using the following Laplace equation,

$$\beta_s = 1/ u^2 \rho. \qquad \text{.....(i)}$$

Compressibility lowering is evaluated as the difference in the compressibility of solvent and solution,

$$\Delta\beta_s = \beta_s^o - \beta_s. \qquad \text{.....(ii)}$$

The apparent molal volumes, V_ϕ, and apparent molal adiabatic compressibility K_ϕ of the amino acid solutions were

determined respectively, from the density, ρ, and adiabatic compressibility, β_s, of the solution using the equation,

$$V_\phi = \frac{1000(\rho_0 - \rho)}{m\rho_0\rho} + \frac{M}{\rho} \qquad \text{..... (iii)}$$

$$K_\phi = \frac{1000\,(\beta_s\rho_0 - \beta_s^{\,0}\rho)}{m\,\rho\rho_0} + \frac{\beta_s M}{\rho}. \qquad \text{.....(iv)}$$

The apparent molal volume at infinite dilution (partial molal volume) is obtained by linear extrapolation using the least-squares fit to the equation,

$$V_\phi = V_\phi^0 + S_v m \qquad \text{..... (v)}$$

where V_ϕ^0 is the partial molal volume and S_v is the experimental slope.

Transfer volumes of amino acids, $V_{\phi tr}^0$ from water to different cosolute mixtures are calculated from

$V_{\phi tr}^0(H_2O \rightarrow H_2O +$ Cosolute) = V_ϕ^0 (in H_2O+ Cosolute) - V_ϕ^0 (in H_2O).

.....(vi)

Results and Discussion

The density data of aspartic acid and glutamic acid in salt solutions for several concentrations and temperatures have been given in Tables 1.1(a-f). The density values have been found to exhibit the usual decrease with an increase in temperature and increase with an increase in concentration.

The measured values of ultrasonic velocities (U) are listed in Tables 1.2(a-f) at different temperatures for each of the composition studied. The values of U are found to increase with temperature and are also affected by change in concentration. The

Table 1.1(a): Densities, ρ (gm cm^{-3}) of Aspartic acid in 1M aqueous CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0097	0.0485	0.0975	0.1471	0.1972
298.15	1.03703	1.03757	1.03972	1.04241	1.04510	1.04779
303.15	1.03471	1.03525	1.03740	1.04009	1.04278	1.04547
308.15	1.03242	1.03296	1.03511	1.03780	1.04049	1.04318
313.15	1.03011	1.03065	1.03280	1.03549	1.03818	1.04087
318.15	1.02784	1.02838	1.03053	1.03322	1.03591	1.03860
323.15	1.02551	1.02605	1.02820	1.03089	1.03358	1.03627

Table 1.1(b): Densities, ρ (gm cm^{-3}) of Glutamic acid in 1M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0097	0.0485	0.0975	0.1472	0.1972
298.15	1.03703	1.03755	1.03961	1.04219	1.04477	1.04734
303.15	1.03471	1.03523	1.03729	1.03987	1.04245	1.04502
308.15	1.03242	1.03294	1.03500	1.03758	1.04016	1.04273
313.15	1.03011	1.03063	1.03269	1.03527	1.03785	1.04042
318.15	1.02784	1.02836	1.03042	1.03300	1.03558	1.03815
323.15	1.02551	1.02603	1.02809	1.03067	1.03325	1.03582

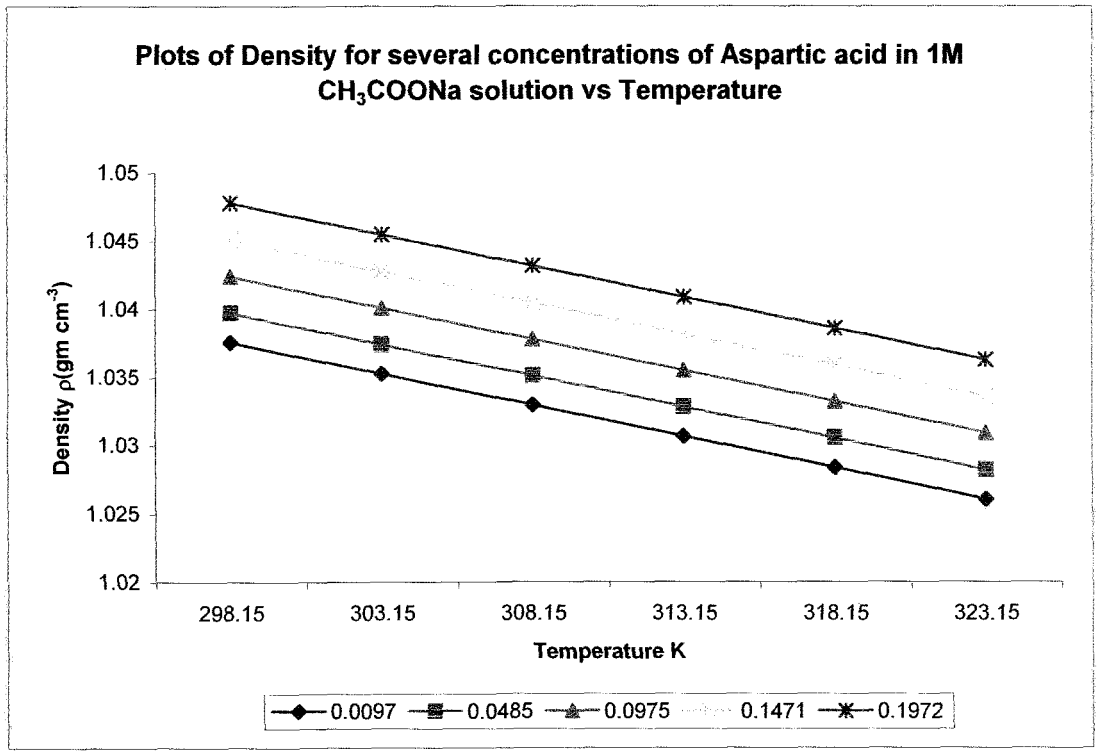


Fig. 1.1(a)

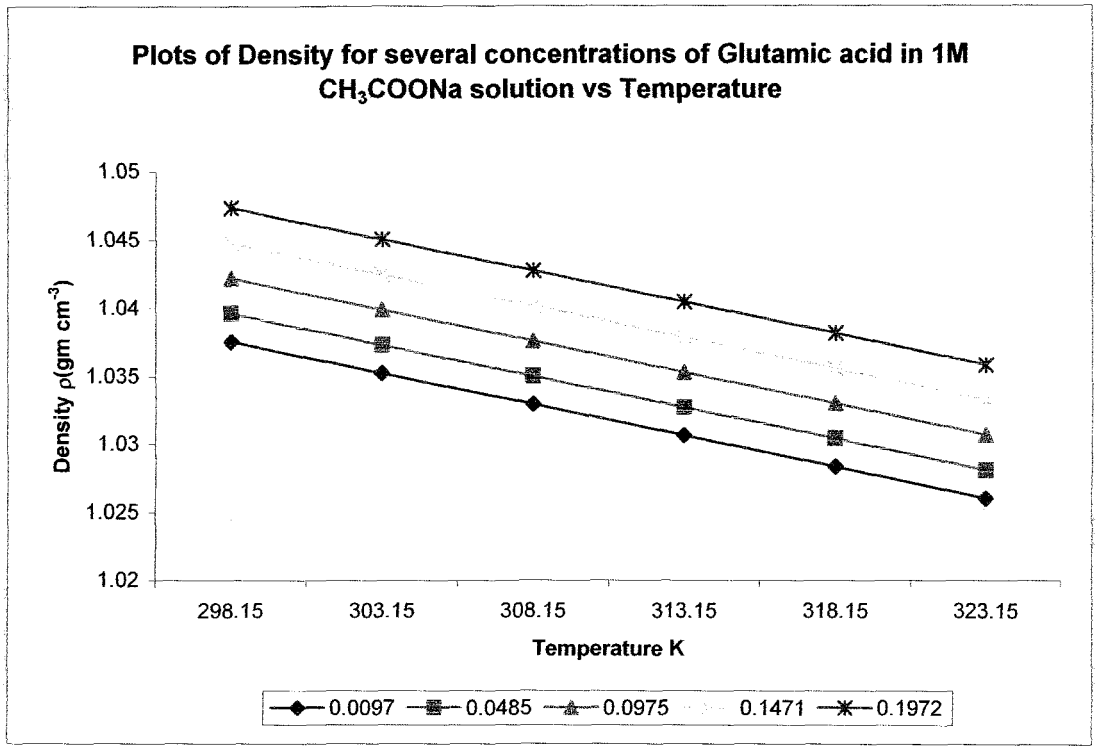


Fig. 1.1(b)

Table 1.1(c): Densities, ρ (gm cm^{-3}) of Aspartic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0094	0.0470	0.0944	0.1425	0.1910
298.15	1.07042	1.07092	1.07291	1.07539	1.07788	1.08037
303.15	1.06904	1.06954	1.07153	1.07401	1.07650	1.07899
308.15	1.06763	1.06813	1.07012	1.07260	1.07509	1.07758
313.15	1.06622	1.06672	1.06871	1.07119	1.07368	1.07617
318.15	1.06481	1.06531	1.06730	1.06978	1.07227	1.07476
323.15	1.06303	1.06353	1.06552	1.06800	1.07049	1.07298

Table 1.1(d): Densities, ρ (gm cm^{-3}) of Glutamic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0094	0.0467	0.0945	0.1426	0.1912
298.15	1.07042	1.07089	1.07274	1.07508	1.07740	1.07971
303.15	1.06904	1.06951	1.07136	1.07370	1.07602	1.07833
308.15	1.06763	1.06810	1.06995	1.07229	1.07461	1.07692
313.15	1.06622	1.06669	1.06854	1.07088	1.07320	1.07551
318.15	1.06481	1.06528	1.06713	1.06947	1.07179	1.07410
323.15	1.06303	1.06350	1.06535	1.06769	1.07001	1.07232

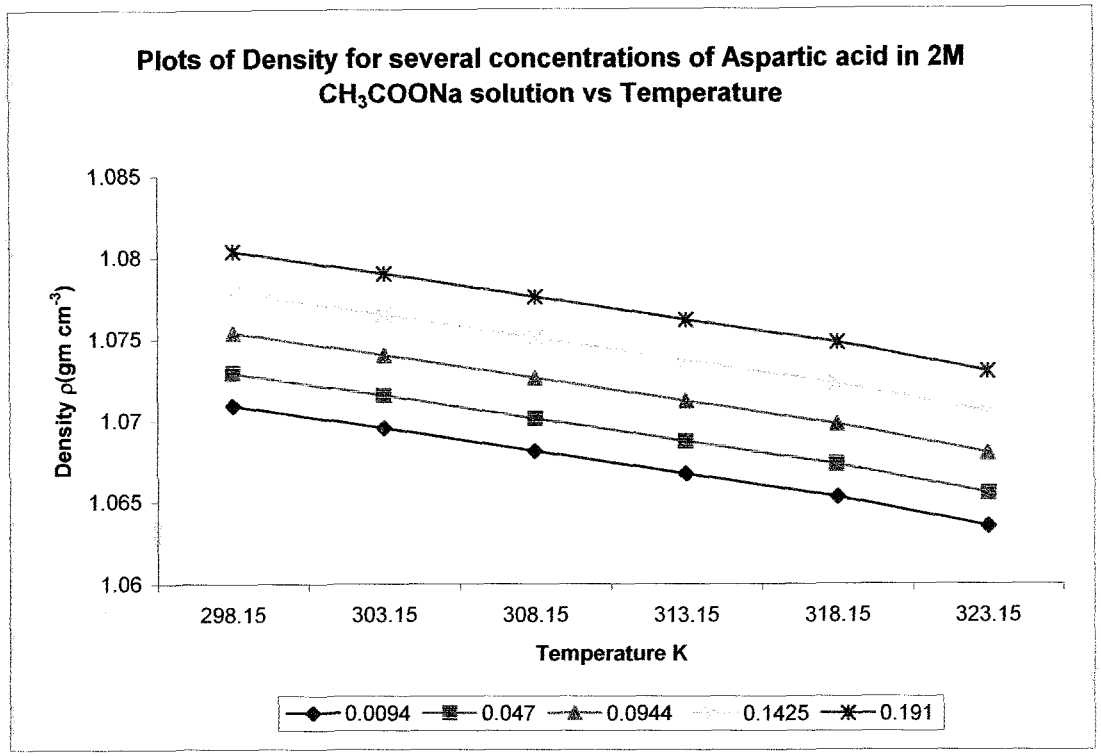


Fig. 1.1(c)

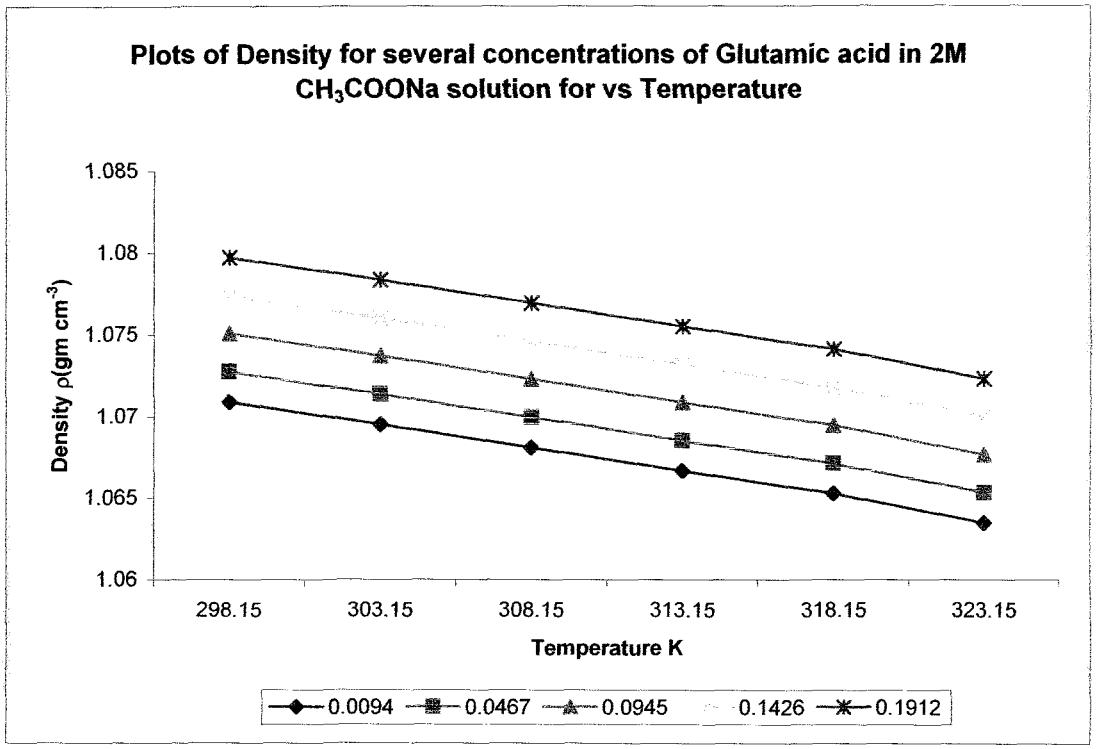


Fig. 1.1(d)

Table 1.1(e): Densities, ρ (gm cm^{-3}) of Aspartic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0096	0.0481	0.0969	0.1461	0.1959
298.15	1.04365	1.04417	1.04624	1.04884	1.05143	1.05403
303.15	1.04140	1.04192	1.04399	1.04659	1.04918	1.05178
308.15	1.03895	1.03947	1.04154	1.04414	1.04673	1.04933
313.15	1.03660	1.03712	1.03919	1.04179	1.04438	1.04698
318.15	1.03425	1.03477	1.03684	1.03944	1.04203	1.04463
323.15	1.03190	1.03242	1.03449	1.03709	1.03968	1.04228

Table 1.1(f): Densities, ρ (gm cm^{-3}) of Glutamic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0096	0.0482	0.0965	0.1461	0.1959
298.15	1.04365	1.04415	1.04614	1.04860	1.05109	1.05356
303.15	1.04140	1.04190	1.04389	1.04635	1.04884	1.05131
308.15	1.03895	1.03945	1.04144	1.04390	1.04639	1.04886
313.15	1.03660	1.03710	1.03909	1.04155	1.04404	1.04651
318.15	1.03425	1.03475	1.03674	1.03920	1.04169	1.04416
323.15	1.03190	1.03240	1.03439	1.03685	1.03934	1.04181

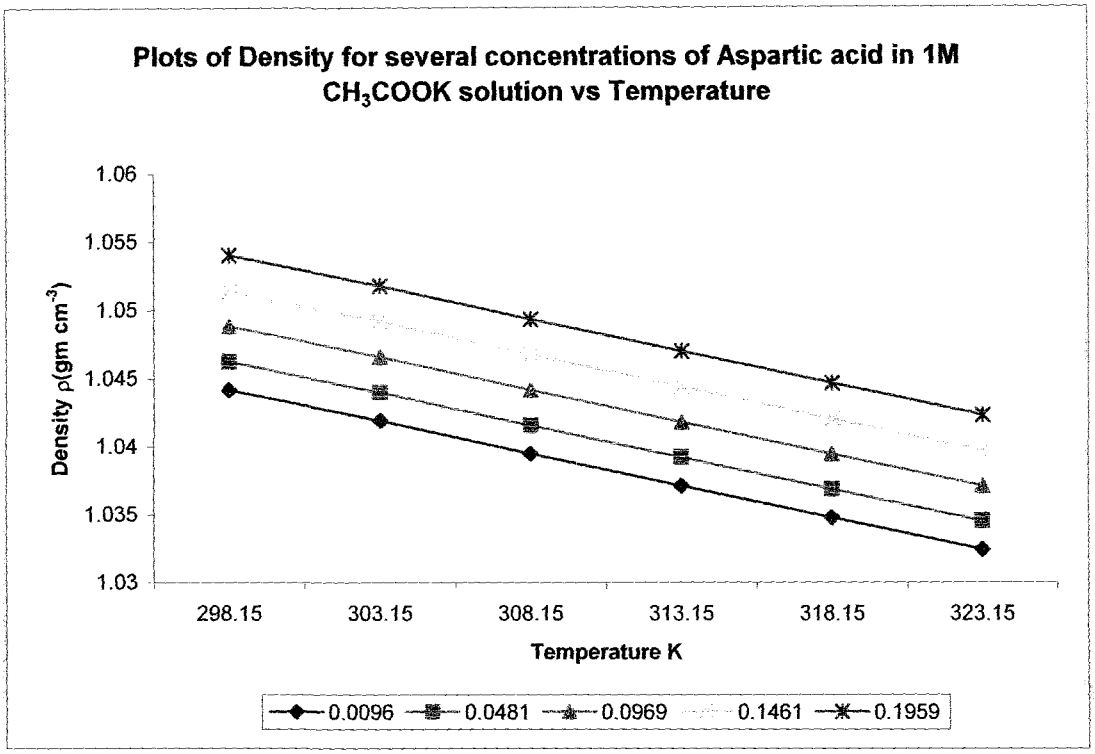


Fig. 1.1(e)

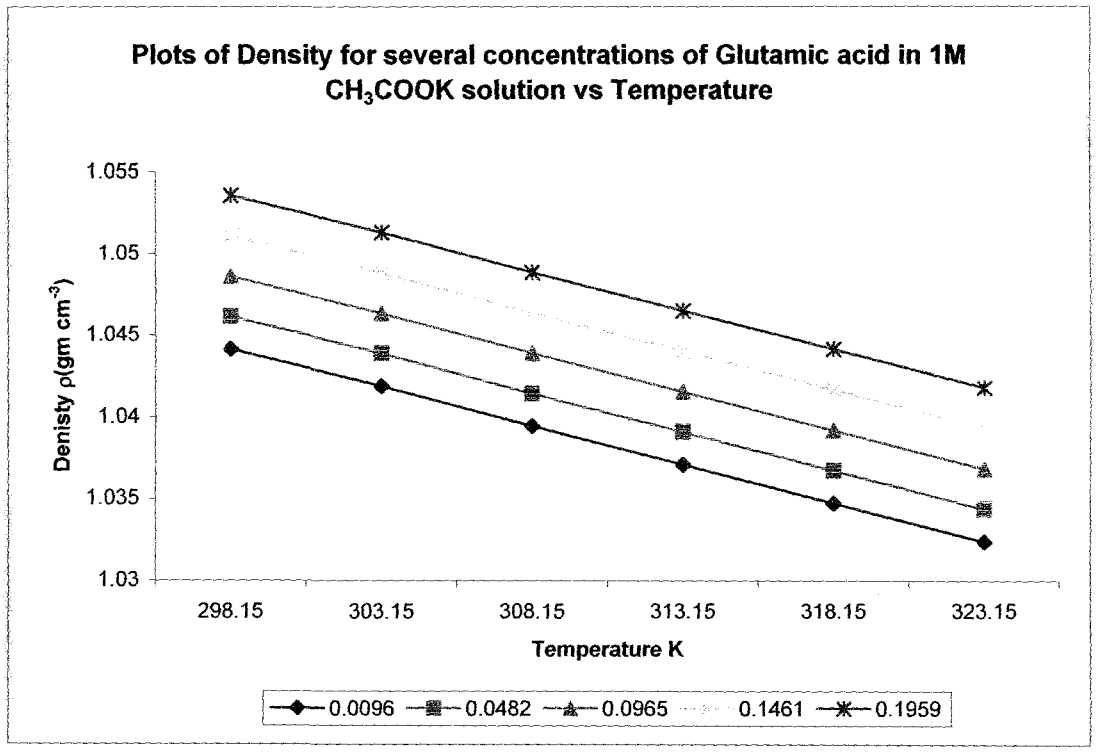


Fig. 1.1(f)

Table 1.2(a): Ultrasonic Velocities, U(ms^{-1}) of Aspartic acid in 1M CH_3COONa solution for different Concentrations and Temperatures:

Molality mol kg^{-1} / Temp. K	0.0000	0.0097	0.0485	0.0975	0.1471	0.1972
303.15	1593.32	1597.36	1599.00	1600.40	1604.20	1602.16
308.15	1599.80	1606.48	1607.16	1606.44	1608.48	1610.60
313.15	1606.60	1610.84	1610.80	1611.72	1614.76	1615.44
318.15	1610.60	1615.72	1615.72	1616.60	1618.40	1621.32
323.15	1613.00	1618.52	1618.64	1620.88	1622.12	1622.56

Table 1.2(b): Ultrasonic Velocities, U(ms^{-1}) of Glutamic acid in 1M CH_3COONa solution for different Concentrations and Temperatures:

Molality mol kg^{-1} / Temp. K	0.0000	0.0097	0.0485	0.0975	0.1472	0.1972
303.15	1593.32	1596.36	1599.00	1602.16	1604.11	1606.64
308.15	1599.80	1601.36	1605.12	1607.36	1610.12	1611.52
313.15	1606.60	1606.90	1613.08	1610.72	1616.40	1616.76
318.15	1610.60	1615.23	1616.52	1618.48	1619.68	1623.12
323.15	1613.00	1616.24	1618.36	1620.40	1622.84	1625.84

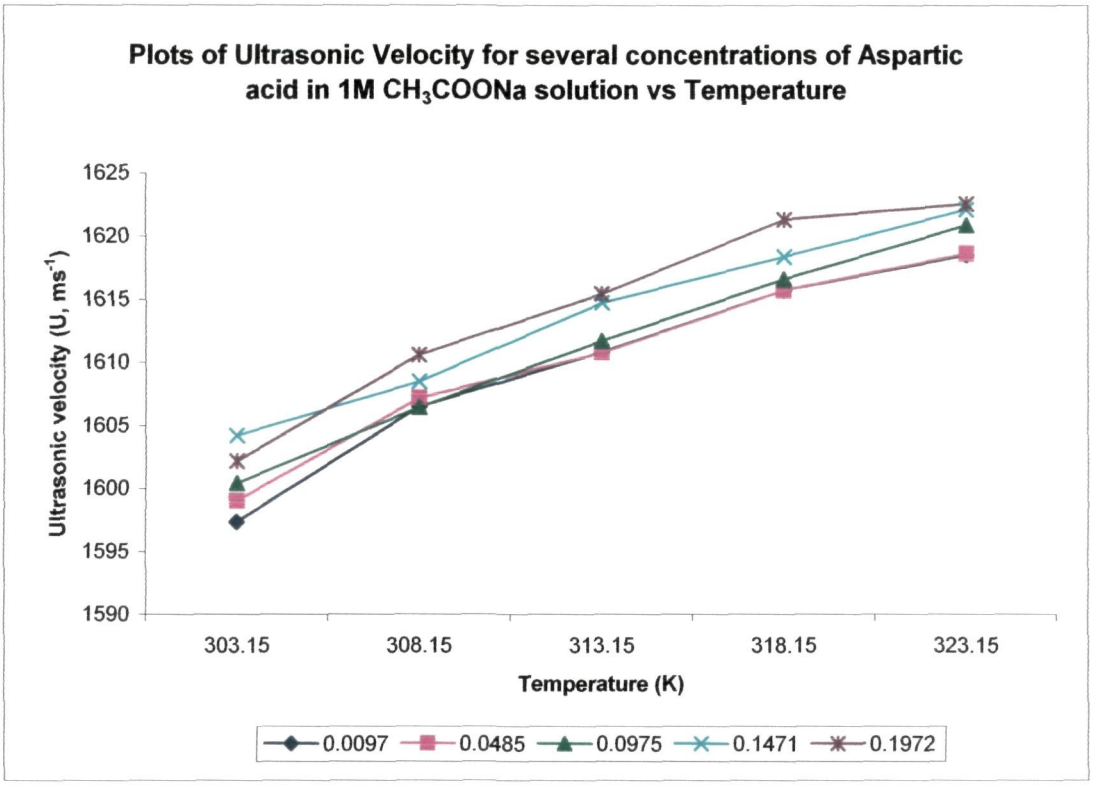


Fig. 1.2(a)

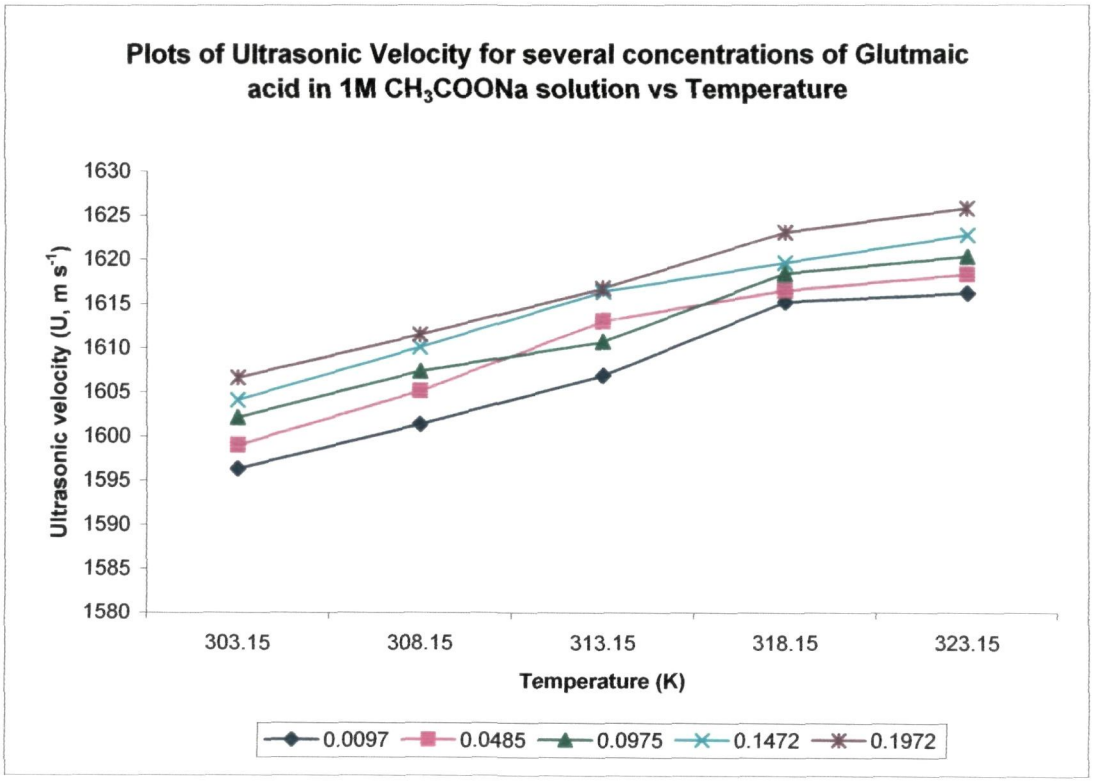


Fig. 1.2(b)

Table 1.2(c): Ultrasonic Velocities, U(ms^{-1}) of Aspartic acid in 2M CH_3COONa solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0000	0.0094	0.0470	0.0944	0.1425	0.1910
303.15	1671.40	1673.28	1675.24	1676.36	1677.36	1677.76
308.15	1674.88	1678.48	1678.64	1679.24	1681.24	1681.92
313.15	1677.84	1680.36	1681.60	1682.24	1683.00	1683.48
318.15	1679.00	1682.56	1682.36	1683.32	1684.56	1684.84
323.15	1681.20	1683.40	1684.48	1685.28	1686.16	1685.40

Table 1.2(d): Ultrasonic Velocities, U(ms^{-1}) of Glutamic acid in 2M CH_3COONa solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0000	0.0094	0.0467	0.0945	0.1426	0.1912
303.15	1671.40	1676.40	1677.50	1678.75	1680.03	1681.36
308.15	1674.88	1680.28	1681.71	1682.66	1683.74	1684.92
313.15	1677.84	1682.76	1683.86	1684.91	1685.77	1686.80
318.15	1679.00	1683.60	1685.00	1686.44	1687.42	1688.68
323.15	1681.20	1684.36	1685.75	1686.95	1688.26	1689.72

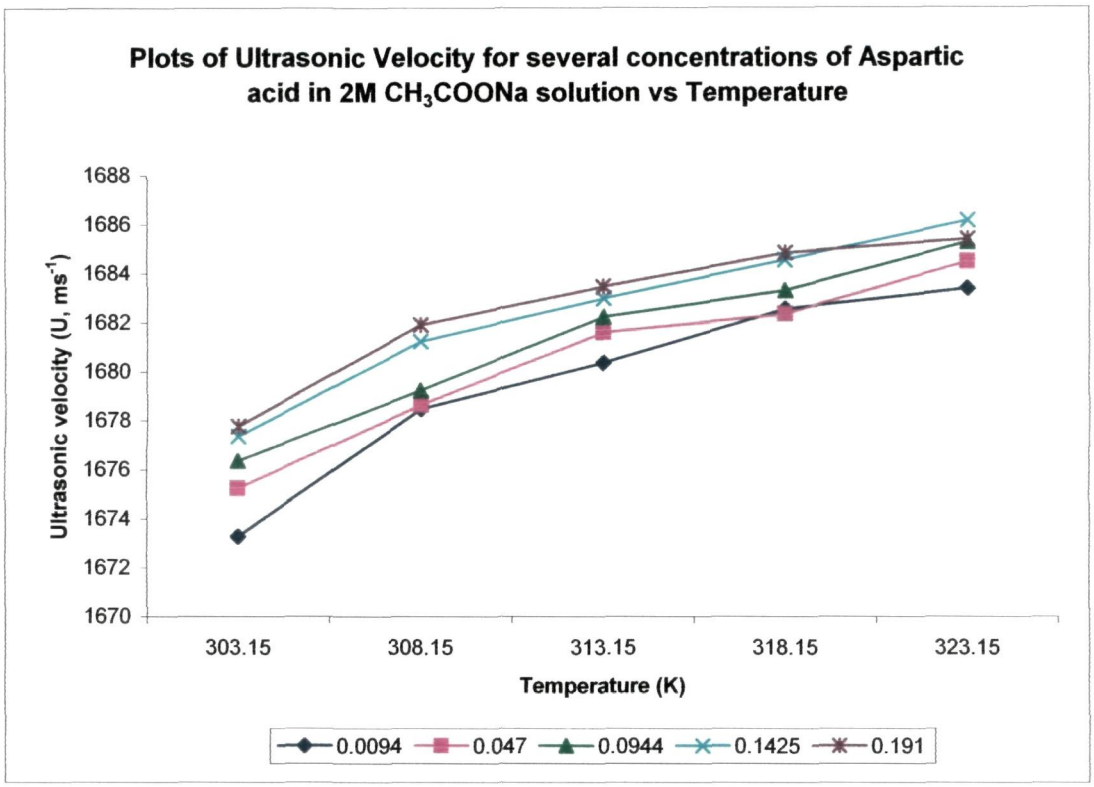


Fig. 1.2(c)

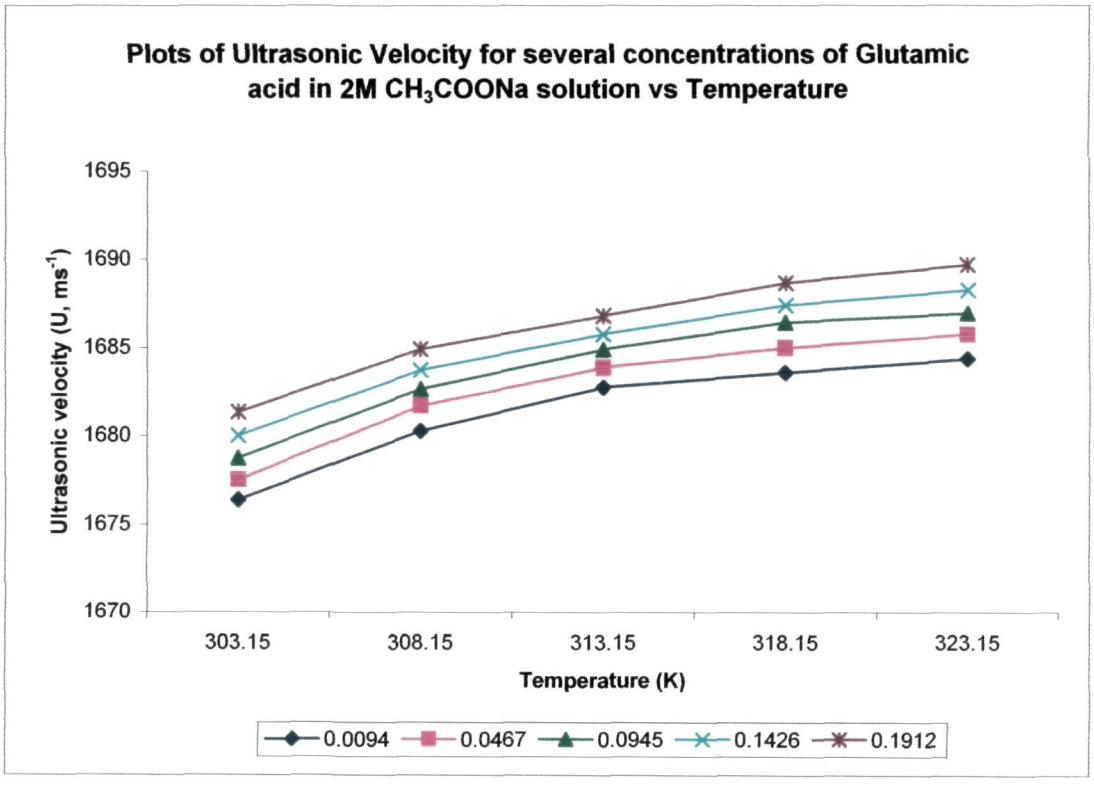


Fig. 1.2(d)

Table 1.2(e): Ultrasonic Velocities, U(ms^{-1}) of Aspartic acid in 1M CH_3COOK solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0000	0.0096	0.0481	0.0969	0.1461	0.1959
303.15	1579.48	1581.28	1581.88	1583.40	1584.68	1588.84
308.15	1586.80	1588.08	1589.16	1590.86	1591.48	1595.80
313.15	1593.28	1594.56	1595.16	1597.88	1599.24	1600.92
318.15	1597.60	1599.40	1600.24	1601.44	1605.16	1606.28
323.15	1602.32	1603.40	1603.04	1606.36	1607.36	1608.60

Table 1.2(f): Ultrasonic Velocities, U(ms^{-1}) of Glutamic acid in 1M CH_3COOK solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0000	0.0096	0.0482	0.0965	0.1461	0.1959
303.15	1579.48	1581.48	1582.80	1587.20	1587.56	1589.36
308.15	1586.80	1588.68	1590.20	1593.60	1594.76	1596.68
313.15	1593.28	1595.44	1596.16	1600.68	1599.32	1602.08
318.15	1597.60	1599.40	1600.00	1603.08	1605.16	1606.00
323.15	1602.32	1602.04	1604.36	1607.24	1606.84	1608.72

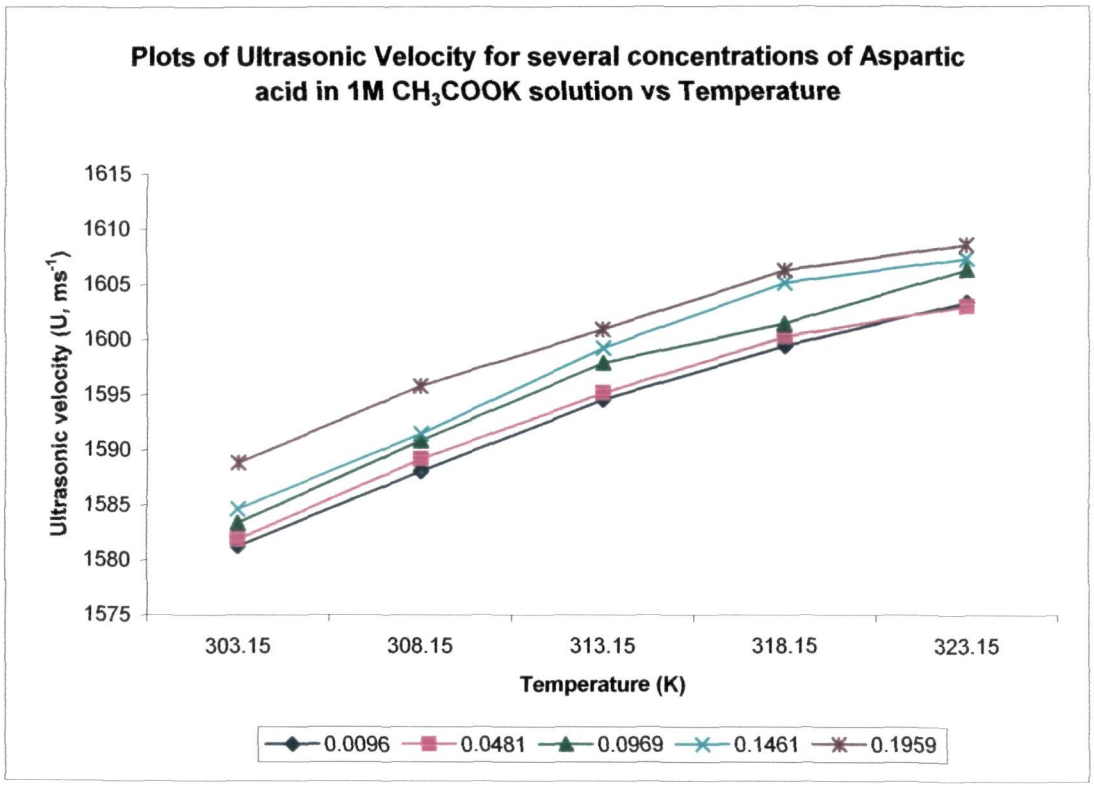


Fig. 1.2(e)

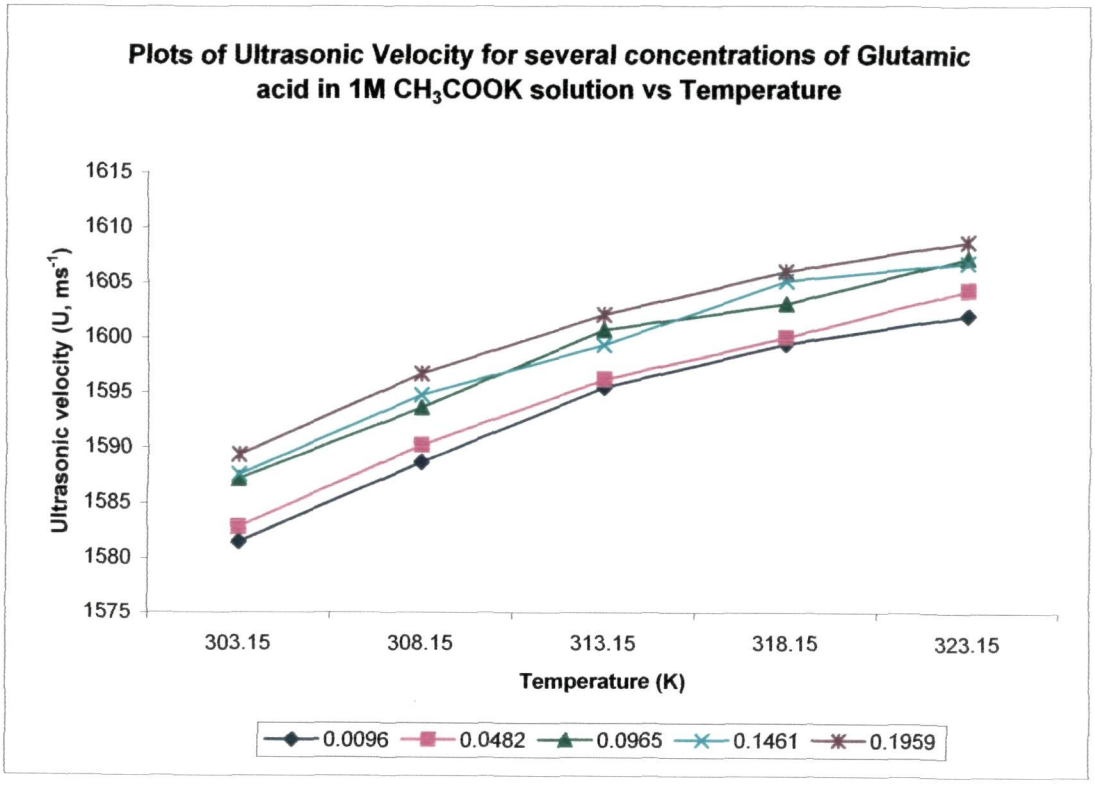


Fig. 1.2(f)

ultrasonic velocities of the said solutions have been found to increase with increase in amino acid concentration. The variation in the values of U with temperature for different composition is shown in Figs. 1.2(a-f). The ultrasonic velocity has been found to increase with increase in concentration of sodium acetate in water for a given amino acid concentration [Tables 1.2(a-c)].

The increase in ultrasonic velocity in these solutions may be attributed to the cohesion brought about by the ionic hydration. When amino acid is dissolved in salt solutions, the cations NH_3^+ and anions COO^- are formed. The water molecules are attached to the ions strongly by the electrostatic forces, which introduce a greater cohesion in the solution. The cohesion in these solutions generally increases with the increase of amino acid concentration. The increased association, observed in these solutions, may also be due to water structure enhancement brought about by the increase in electrostriction in the presence of salt. The electrostriction effect, which brings about the shrinkage in the volume of solvent, caused by the zwitterionic portion of the amino acid, is increased in mixed solvents as compared to that in pure water. This effect is similar to the results of Dash et al [16], Sandu et al [17] and Kaulgud et al [18]. The decrease in adiabatic compressibility [Tables 1.3(a-f)], observed in aqueous salt solutions with aspartic and glutamic acid in the present study generally confirms the conclusion drawn in the velocity studies.

The adiabatic compressibility, β_s, is primarily the compressibility, which increases in thermal breaking of the solvent components, which in turn, results in greater attractive

Table 1.3(a): Adiabatic Compressibility ($\beta_s \times 10^{-7}$, cm^2 $dyne^{-1}$) of Aspartic acid in 1M CH_3COONa solution for different Concentrations and Temperatures:

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1471	0.1972
303.15	3.7857	3.7701	3.7538	3.7264	3.7263
308.15	3.7512	3.7402	3.7339	3.7148	3.6954
313.15	3.7392	3.7316	3.7177	3.6941	3.6815
318.15	3.7249	3.7171	3.7034	3.6856	3.6628
323.15	3.7204	3.7121	3.6922	3.6770	3.6654

Table 1.3(b): Adiabatic Compressibility ($\beta_s \times 10^{-7}$, cm^2 $dyne^{-1}$) of Glutamic acid in 1M CH_3COONa solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1472	0.1972
303.15	3.7905	3.7705	3.7464	3.7280	3.7071
308.15	3.7753	3.7501	3.7304	3.7084	3.6928
313.15	3.7577	3.7215	3.7231	3.6878	3.6771
318.15	3.7272	3.7138	3.6956	3.6809	3.6563
323.15	3.7310	3.7138	3.6952	3.6749	3.6522

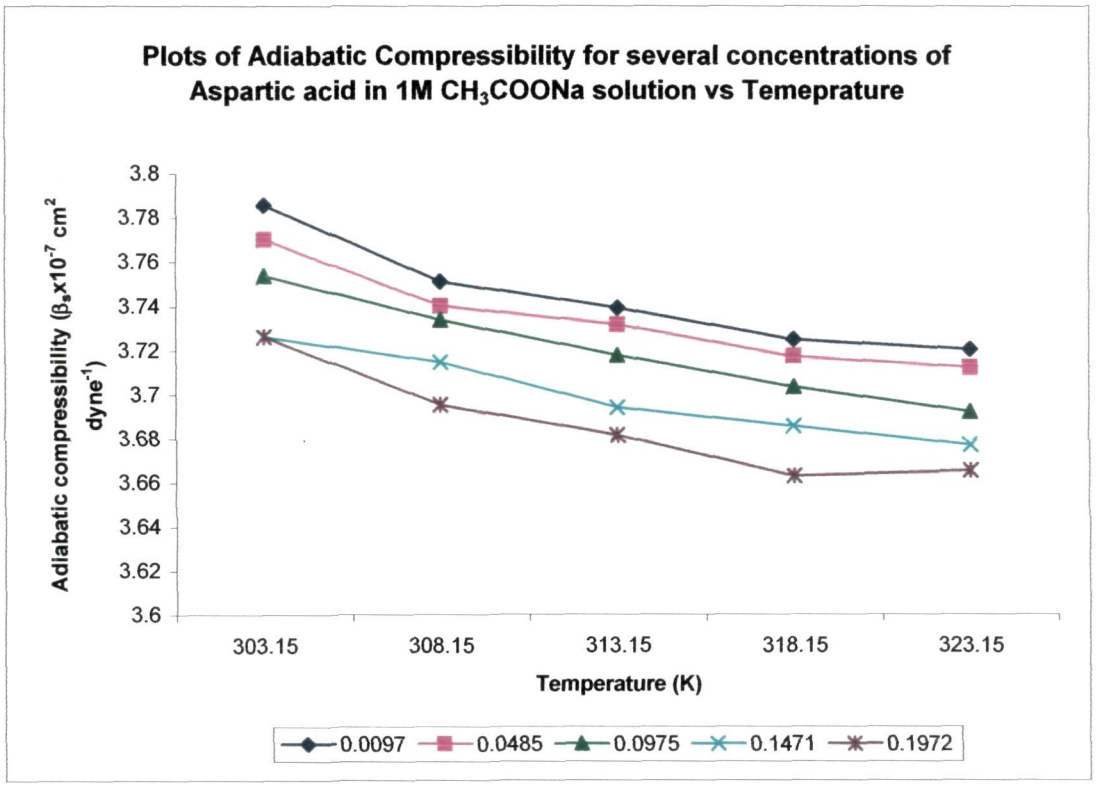


Fig. 1.3(a)

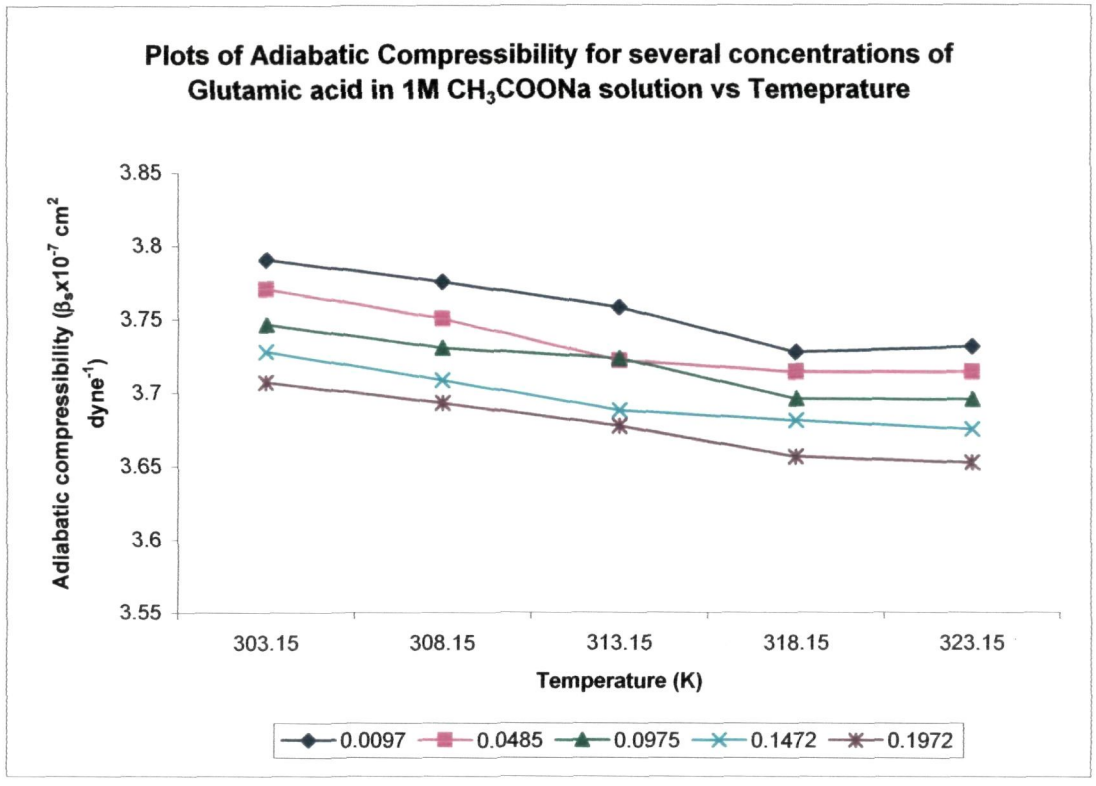


Fig. 1.3(b)

Table 1.3(c): Adiabatic Compressibility ($\beta_s \times 10^{-7}$, cm^2 $dyne^{-1}$) of Aspartic acid in 2M CH_3COONa solution for different Concentrations and Temperatures:

Molality mol kg^{-1} / Temp. K	0.0094	0.0470	0.0944	0.1425	0.1910
303.15	3.3394	3.3254	3.3133	3.3017	3.2925
308.15	3.3231	3.3163	3.3063	3.2908	3.2805
313.15	3.3201	3.3090	3.2988	3.2882	3.2787
318.15	3.3158	3.3104	3.2989	3.2864	3.2777
323.15	3.3180	3.3076	3.2967	3.2856	3.2810

Table 1.3(d): Adiabatic Compressibility ($\beta_s \times 10^{-7}$, cm^2 $dyne^{-1}$) of Glutamic acid in 2M CH_3COONa solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0094	0.0467	0.0945	0.1426	0.1912
303.15	3.3271	3.3170	3.3048	3.2927	3.2804
308.15	3.3161	3.3047	3.2938	3.2825	3.2708
313.15	3.3107	3.3006	3.2893	3.2789	3.2678
318.15	3.3118	3.3005	3.2877	3.2768	3.2648
323.15	3.3143	3.3031	3.2912	3.2789	3.2662

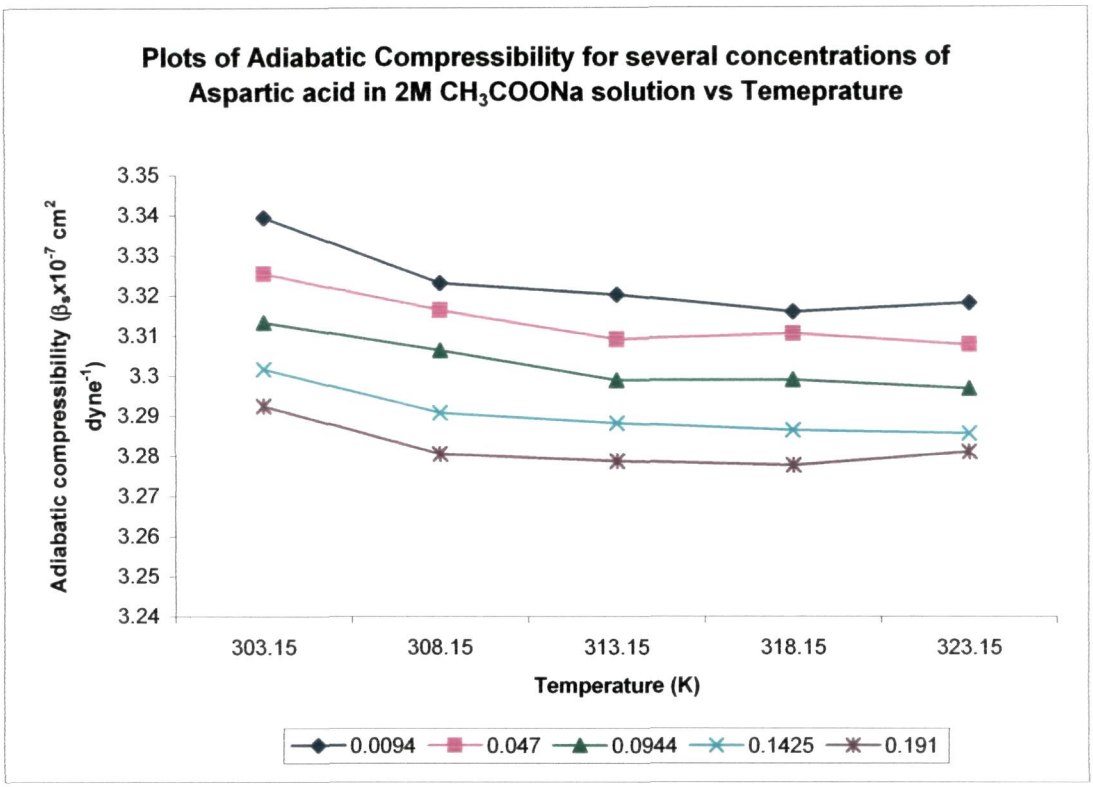


Fig. 1.3(c)

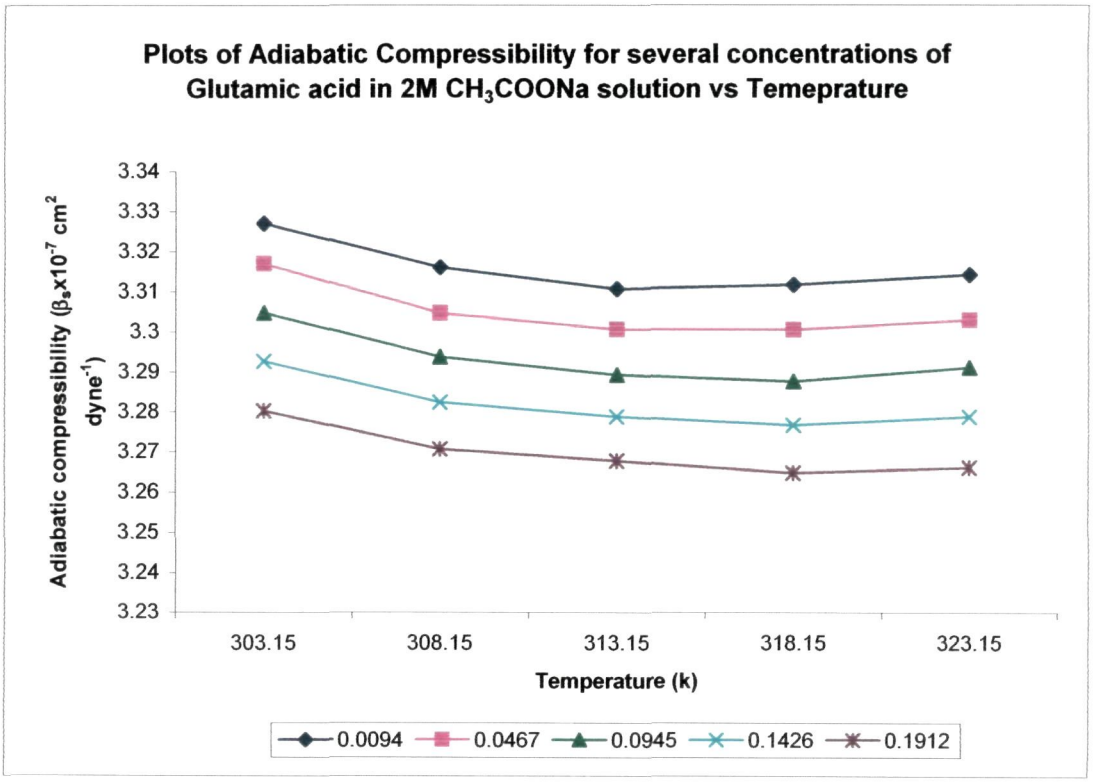


Fig. 1.3(d)

Table 1.3(e): Adiabatic Compressibility ($\beta_s \times 10^{-7}$, cm^2 $dyne^{-1}$) of Aspartic acid in 1M CH_3COOK solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0096	0.0481	0.0969	0.1461	0.1959
303.15	3.8384	3.8279	3.8110	3.7955	3.7663
308.15	3.8145	3.8018	3.7845	3.7719	3.7422
313.15	3.7922	3.7818	3.7595	3.7438	3.7267
318.15	3.7778	3.7663	3.7513	3.7246	3.7102
323.15	3.7676	3.7617	3.7368	3.7228	3.7078

Table 1.3(f): Adiabatic Compressibility ($\beta_s \times 10^{-7}$, cm^2 $dyne^{-1}$) of Glutamic acid in 1M CH_3COOK solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0096	0.0482	0.0965	0.1461	0.1959
303.15	3.8375	3.8238	3.7937	3.7829	3.7655
308.15	3.8117	3.7972	3.7721	3.7576	3.7398
313.15	3.7881	3.7774	3.7472	3.7447	3.7230
318.15	3.7779	3.7678	3.7445	3.7258	3.7131
323.15	3.7740	3.7559	3.7336	3.7265	3.7089

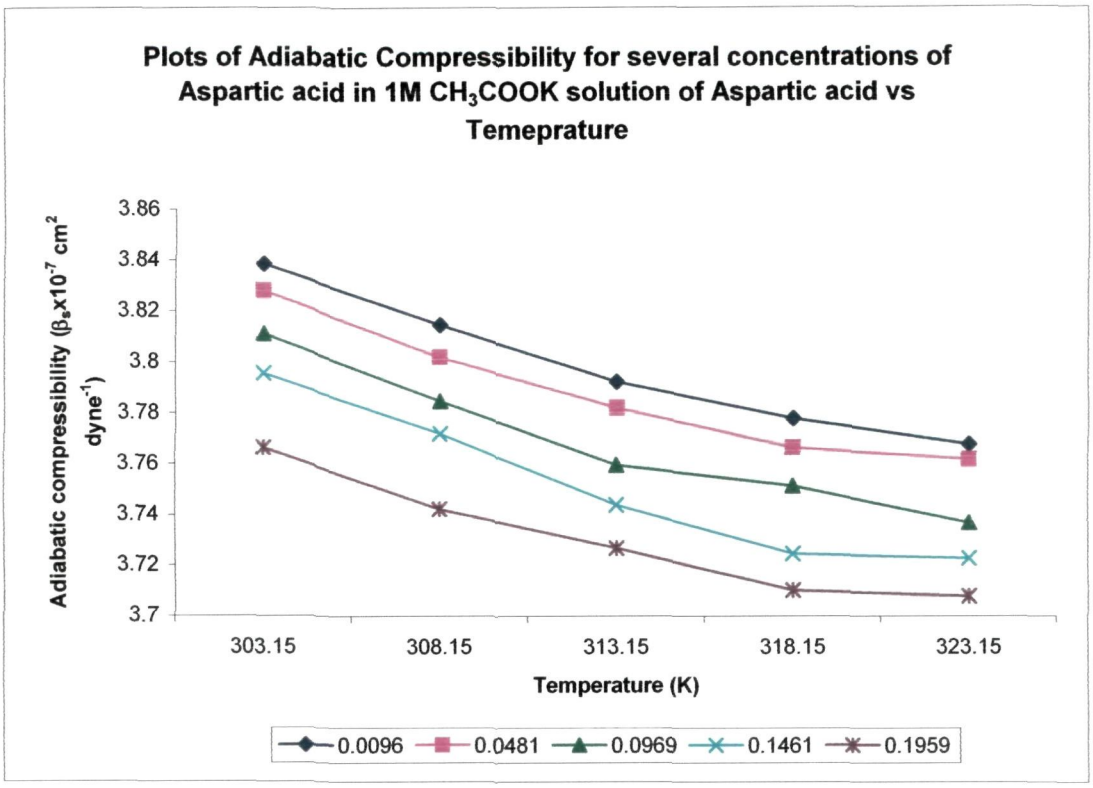


Fig. 1.3(e)

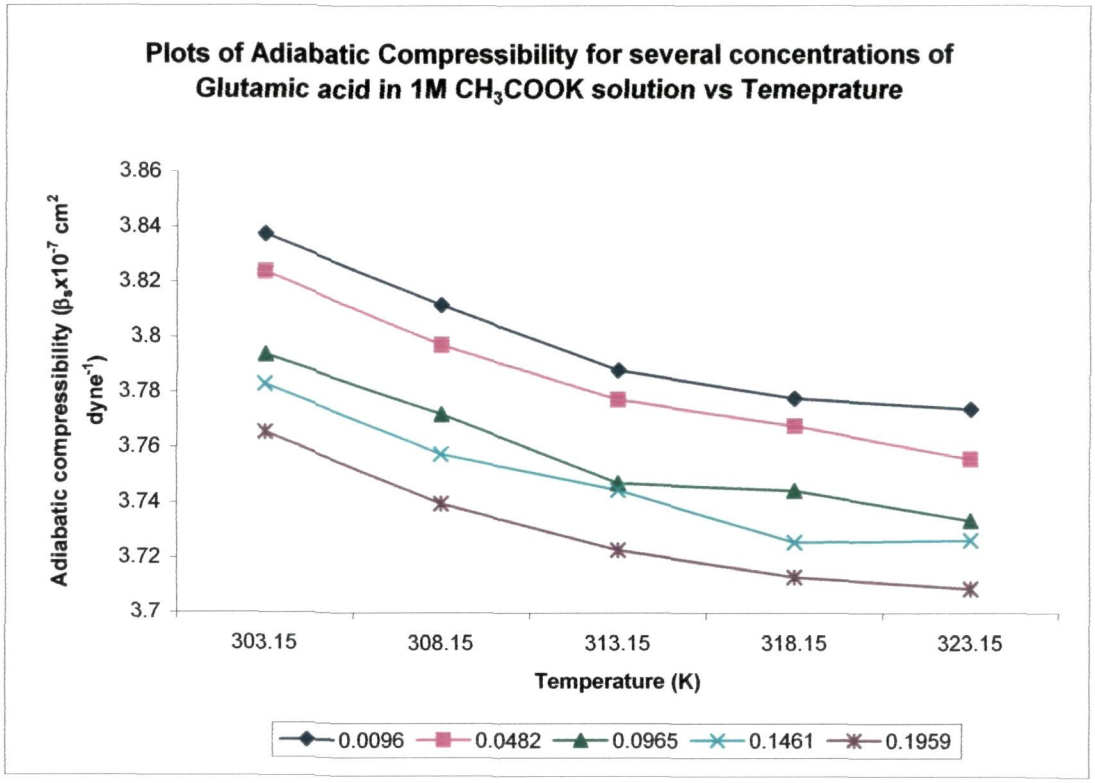


Fig. 1.3(f)

forces among the molecules of a solution. Decrease in the β_s values with increase in concentration [Figs. 1.3(a-f)], may be due to increase in the solute-solvent interactions, which leads to a change in the ultrasonic velocity. The greater the attractive forces among the molecules of a liquid, the smaller will be the compressibility.

Figs. 1.4(a-f) represent the variation of compressibility lowering with temperature for different concentrations of solute. All the figures exhibit linear relationships of compressibility lowering with solute concentration and temperature. The compressibility lowering increases as the concentration of amino acid is increased but it decreases as the concentration of sodium acetate is increased. The values of compressibility lowering are lower in 1M potassium acetate than in 1M sodium acetate. It means that as the size of cation increases in cosolute, compressibility lowering decreases.

It has been observed that after addition of solutes to the salt solutions, there is an increase in the apparent molal adiabatic compressibility (K_ϕ) of the solutions. K_ϕ values of aqueous salt solutions with amino acids at different temperatures are evaluated as shown in Tables 1.5(a-f) and are found to be negative at all temperatures, which can be explained by postulating that COO^- groups of amino acids interact with surrounding water by hydrophilic interactions in such a way that the surrounding water loses its own compressibility to a certain extent and degree of organization of water molecules increases in the vicinity of amino acid. So it will be less compressible.

Table 1.4(a): Compressibility Lowering ($\Delta\beta_s \times 10^{-9}$, cm^2 $dyne^{-1}$) of Aspartic acid in 1M CH_3COONa solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1471	0.1972
303.15	2.1174	3.6766	5.3093	8.0493	8.0616
308.15	3.3338	4.4296	5.0646	6.9740	8.9067
313.15	2.1754	2.9353	4.3295	6.6877	7.9525
318.15	2.5704	3.3475	4.7188	6.5017	8.7792
323.15	2.7451	3.5781	5.5694	7.0931	8.2465

Table 1.4(b): Compressibility Lowering ($\Delta\beta_s \times 10^{-9}$, cm^2 $dyne^{-1}$) of Glutamic acid in 1M CH_3COONa solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1472	0.1972
303.15	1.6357	3.6366	6.0543	7.8942	9.9766
308.15	0.9239	3.4385	5.4128	7.6127	9.1692
313.15	0.3322	3.9498	3.7887	7.3197	8.3944
318.15	2.3360	3.6757	5.5000	6.9667	9.4327
323.15	1.6874	3.4099	5.2717	7.3020	9.5653

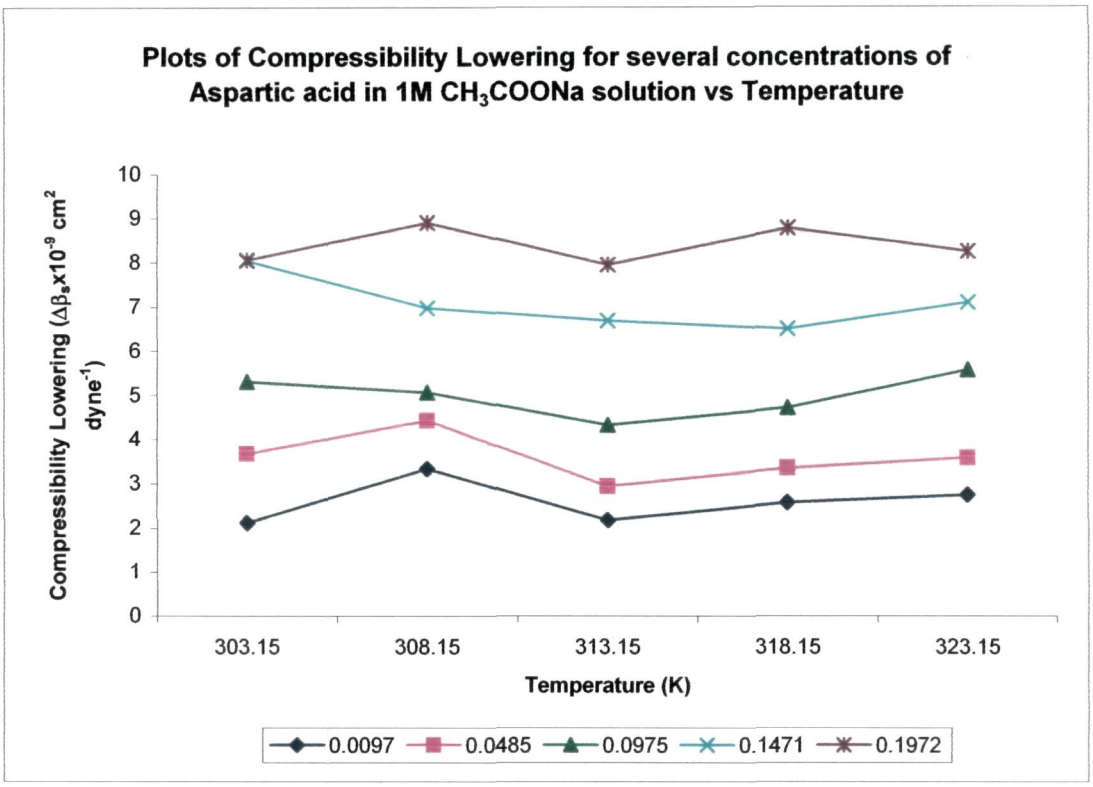


Fig. 1.4(a)

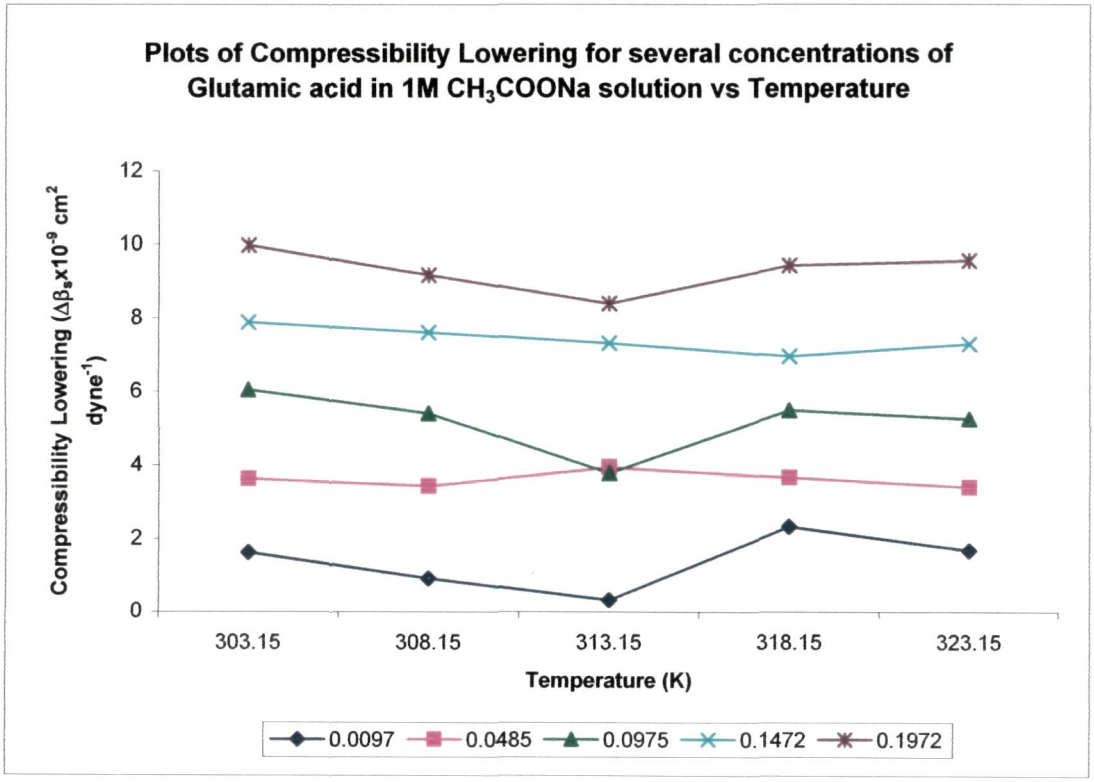


Fig. 1.4(b)

Table 1.4(c): Compressibility Lowering ($\Delta\beta_s \times 10^{-9}$, $cm^2\ dyne^{-1}$) of Aspartic acid in 2M CH_3COONa solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0094	0.0470	0.0944	0.1425	0.1910
303.15	1.0321	2.4318	3.6428	4.8032	5.7222
308.15	1.5899	2.2711	3.2743	4.8243	5.8502
313.15	1.1548	2.2616	3.2788	4.3410	5.2889
318.15	1.5642	2.1037	3.2477	4.4981	5.3685
323.15	1.0206	2.0648	3.1461	4.2561	4.7229

Table 1.4(d): Compressibility Lowering ($\Delta\beta_s \times 10^{-9}$, $cm^2\ dyne^{-1}$) of Glutamic acid in 2M CH_3COONa solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0094	0.0467	0.0945	0.1426	0.1912
303.15	2.2646	3.1507	4.3663	5.5811	6.9299
308.15	2.2922	3.4247	4.5181	5.6507	6.8134
313.15	2.0918	3.0961	4.2277	5.2736	6.3778
318.15	1.9644	3.0874	4.3717	5.4643	6.6570
323.15	1.3894	2.5151	3.7077	4.9308	6.1975

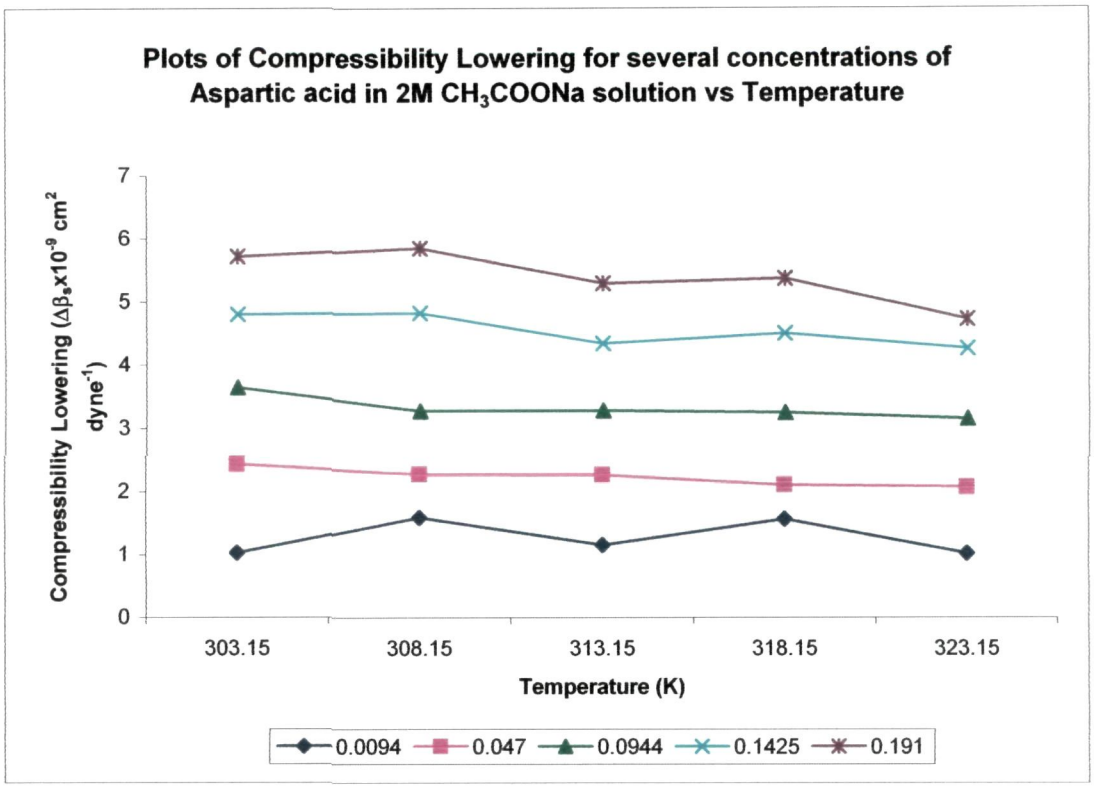


Fig. 1.4(c)

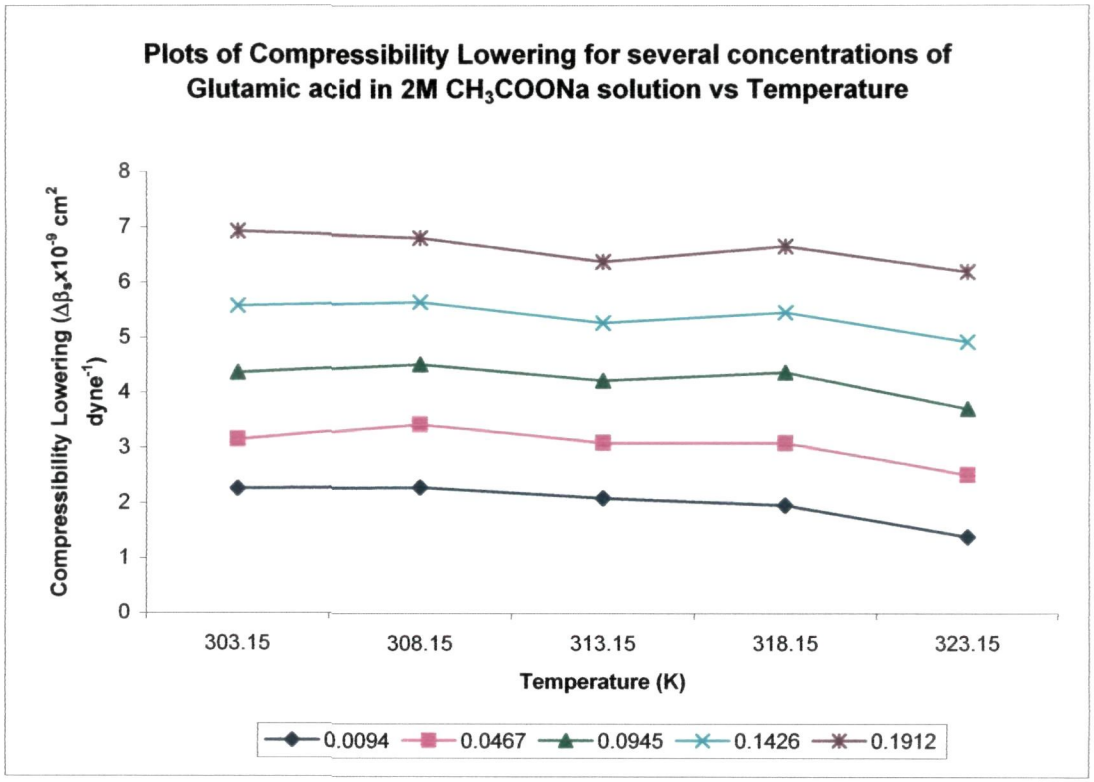


Fig. 1.4(d)

Table 1.4(e): Compressibility Lowering ($\Delta\beta_s \times 10^{-9}$, $cm^2\ dyne^{-1}$) of Aspartic acid in 1M CH_3COOK solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0096	0.0481	0.0969	0.1461	0.1959
303.15	1.1019	2.1535	3.8372	5.3919	8.3101
308.15	0.8050	2.0804	3.8086	5.0676	8.0366
313.15	0.7517	1.7918	4.0188	5.5887	7.3018
318.15	1.0374	2.1873	3.6922	6.3570	7.8020
323.15	0.6943	1.2793	3.7718	5.1663	6.6673

Table 1.4(f): Compressibility Lowering ($\Delta\beta_s \times 10^{-9}$, $cm^2\ dyne^{-1}$) of Glutamic acid in 1M CH_3COOK solution for different Concentrations and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0096	0.0482	0.0965	0.1461	0.1959
303.15	1.1916	2.5618	5.5729	6.6451	8.3883
308.15	1.0858	2.5411	5.0506	6.4955	8.2814
313.15	1.1626	2.2291	5.2466	5.5042	7.6741
318.15	1.0301	2.0427	4.3729	6.2354	7.5055
323.15	0.0471	1.8617	4.0945	4.8035	6.5554

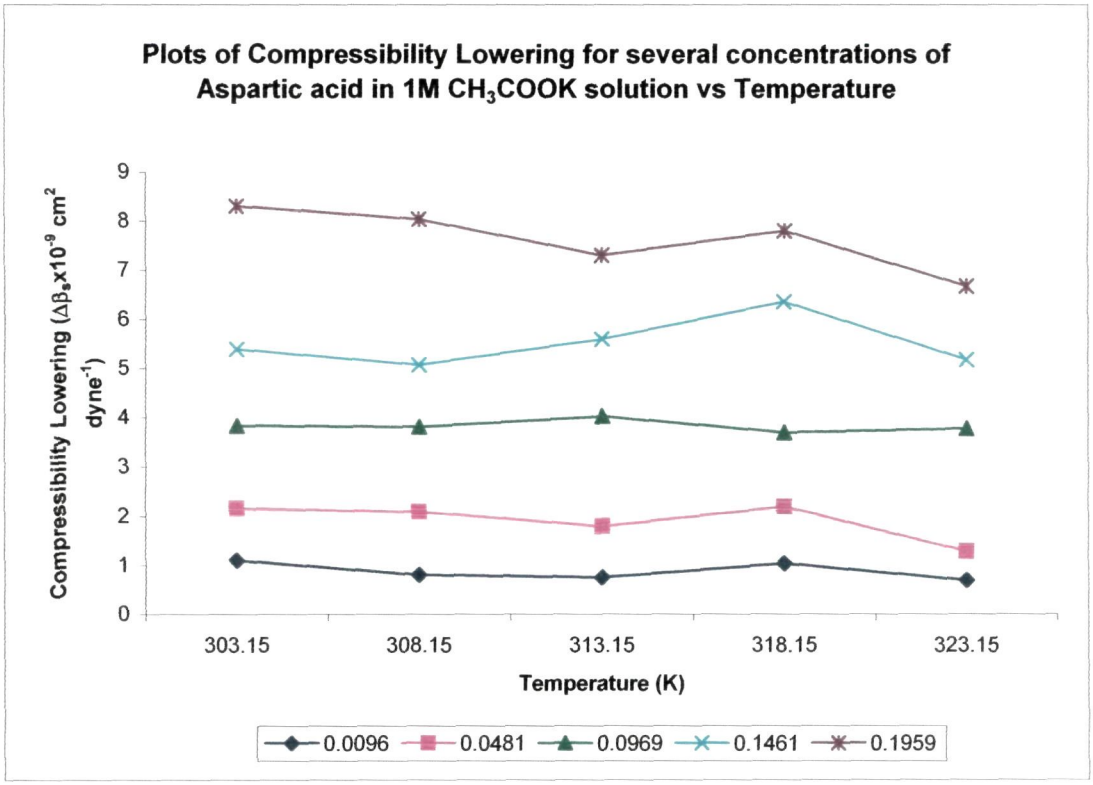


Fig. 1.4(e)

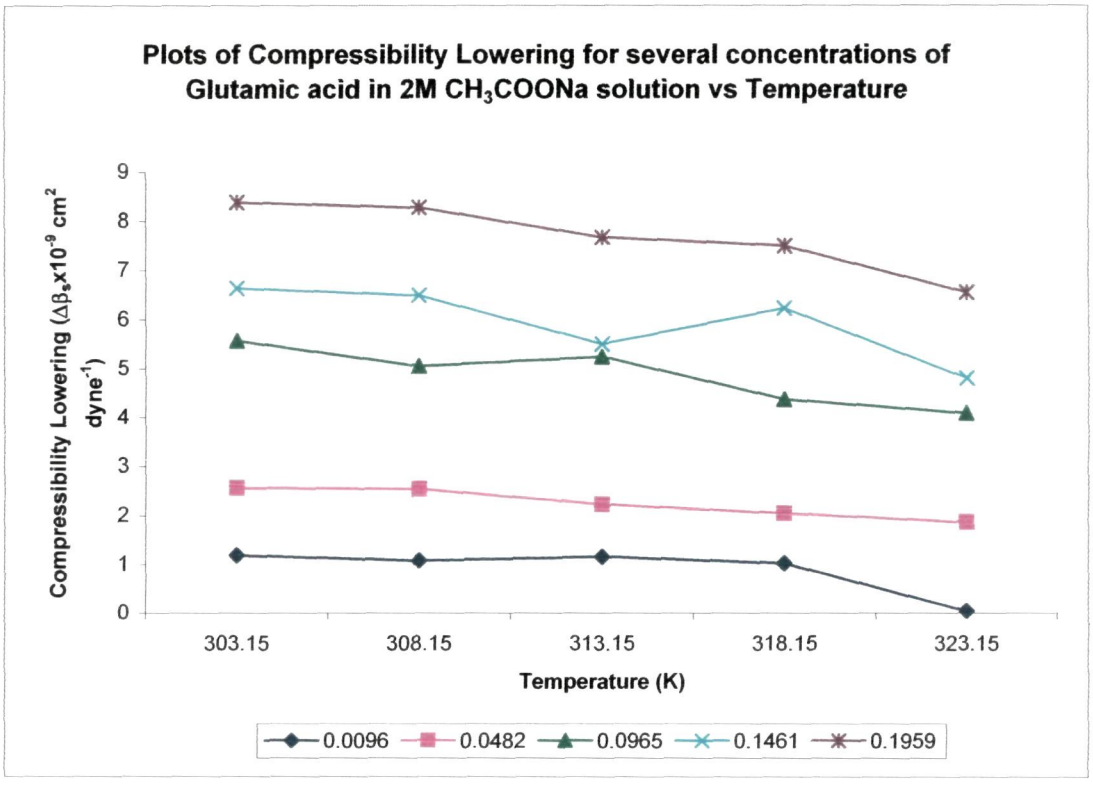


Fig. 1.4(f)

Table 1.5(a): Apparent Molal Adiabatic Compressibility ($K_\phi \times 10^{-5}$, $cm^3 mol^{-1} dyne^{-1}$) of Aspartic acid in 1M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1471	0.1972
303.15	-18.1971	-4.4372	-2.3837	-2.4268	-1.0862
308.15	-30.4142	-5.9783	-2.1656	-1.7373	-1.5319
313.15	-18.9035	-3.0116	-1.4553	-1.5724	-1.0807
318.15	-22.9218	-3.8606	-1.8622	-1.4637	-1.5095
323.15	-24.7384	-4.3413	-2.7302	-1.8701	-1.2518

Table 1.5(b): Apparent Molal Adiabatic Compressibility ($K_\phi \times 10^{-5}$, $cm^3 mol^{-1} dyne^{-1}$) of Glutamic acid in 1M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1471	0.1972
303.15	-12.8066	-3.7672	-2.5433	-1.7387	-1.4626
308.15	-5.7463	-3.4029	-1.9306	-1.5796	-1.0865
313.15	0.1419	-4.4648	-0.3287	-1.4131	-0.7263
318.15	-19.9989	-3.9360	-2.0666	-1.1933	-1.2634
323.15	13.5142	-3.4149	-1.8477	-1.4279	-1.3398

Table 1.5(c): Apparent Molal Adiabatic Compressibility ($K_\phi \times 10^{-5}$, $cm^3 mol^{-1} dyne^{-1}$) of Aspartic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0094	0.0470	0.0944	0.1425	0.1910
303.15	-6.4742	-2.0083	-0.9040	-0.4939	-0.2360
308.15	-13.2515	-1.9391	-0.6661	-0.5979	-0.3024
313.15	-8.9322	-1.9303	-0.6793	-0.2846	-0.0305
318.15	-13.0393	-1.6185	-0.6512	-0.3919	0.0725
323.15	-7.6222	-1.5481	0.5553	-0.2360	0.2453

Table 1.5(d): Apparent Molal Adiabatic Compressibility ($K_\phi \times 10^{-5}$, $cm^3 mol^{-1} dyne^{-1}$) of Glutamic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0094	0.0470	0.0944	0.1425	0.1910
303.15	-18.2195	-3.2022	-1.2170	-.05625	-0.2384
308.15	-19.7263	-3.7689	-1.3811	-0.6202	-0.2531
313.15	-17.7605	-3.1137	-1.09878	-0.3763	-0.0427
318.15	-16.5113	-3.1016	-1.2455	-0.5061	-0.1846
323.15	-10.7846	-1.9438	-0.5807	-0.1520	0.0401

Table 1.5(e): Apparent Molal Adiabatic Compressibility ($K_\phi \times 10^{-5}$, $cm^3 mol^{-1} dyne^{-1}$) of Aspartic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0096	0.0481	0.0969	0.1461	0.1959
303.15	-8.0347	-1.3148	-0.8287	-0.5787	-1.1292
308.15	-5.1001	-1.1965	-0.8274	-0.3892	-1.0206
313.15	-4.5979	-0.6402	-1.0623	0.7604	-0.6775
318.15	-7.5008	-1.4532	-0.7496	-1.2899	-0.9430
323.15	-4.0671	0.3651	-0.8465	-0.5087	-0.3900

Table 1.5(f): Apparent Molal Adiabatic Compressibility ($K_\phi \times 10^{-5}$, $cm^3 mol^{-1} dyne^{-1}$) of Glutamic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0096	0.0482	0.0965	0.1461	0.1959
303.15	-8.3423	-1.5312	-1.9969	-0.8246	-0.5817
308.15	-7.3290	-1.5227	-1.5052	-0.7559	-0.5590
313.15	-8.1444	-0.9245	-1.7319	-0.1193	-0.2810
318.15	-6.8414	-0.5563	-0.8671	-0.6252	-0.2117
323.15	3.0584	-0.2186	-0.6038	-0.3197	0.2499

The experimental values of apparent molal volume, V_ϕ, calculated from the experimental density values are given in Tables 1.6(a-f). The V_ϕ values, which give information about the solute-solute and solute-solvent interactions, are positive for all the compositions and temperatures.

Partial molal volume, V_ϕ^0, has been calculated by the least-squares method using equation (v) in which S_v is the experimental slope. The measured partial molal volume can be considered to be a sum of the geometric volume of the solute molecules and change in the solvent due to its interaction with the solute [19-22]. The values of S_v are positive and increase with increase in temperature and also increase as the number of methylene group increases.

Volumetric studies of amino acids and other zwitterions reported in literature represent some important features of dipolar ion hydration [23-31], summarized as follows:

(i) NH_3^+ and COO^- terminals, being charged ends produce strong electrostrictive compression around the solvent, while the intervening part of the molecule interacts with the solvent in a manner that depends largely on whether the residue is hydrophobic, hydrophilic or amphiphilic.

(ii) The electrostrictive compression due to NH_3^+ group is higher than that of COO^- group by about 10 times.

(iii) The overlap of hydration cosphere of terminal (NH_3^+ and COO^-) groups and of the group adjacent to them results in a volume change.

Table 1.6(a): Apparent Molal Volume V_ϕ (cm^3 mol^{-1}) of Aspartic acid in 1M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1471	0.1972
298.15	76.54	76.58	76.64	76.74	76.81
303.15	76.60	76.63	76.70	76.79	76.87
308.15	76.65	76.69	76.75	76.85	76.93
313.15	76.71	76.74	76.81	76.91	76.98
318.15	76.76	76.79	76.86	76.96	77.04
323.15	76.81	76.85	76.92	77.02	77.10

Table 1.6(b): Apparent Molal Volume V_ϕ (cm^3 mol^{-1}) of Glutamic acid in 1M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1472	0.1972
298.15	91.98	92.18	92.21	92.29	92.34
303.15	92.08	92.28	92.30	92.39	92.44
308.15	92.17	92.37	92.40	92.49	92.54
313.15	92.26	92.47	92.49	92.58	92.63
318.15	92.35	92.60	92.59	92.68	92.73
323.15	92.45	92.65	92.68	92.77	92.82

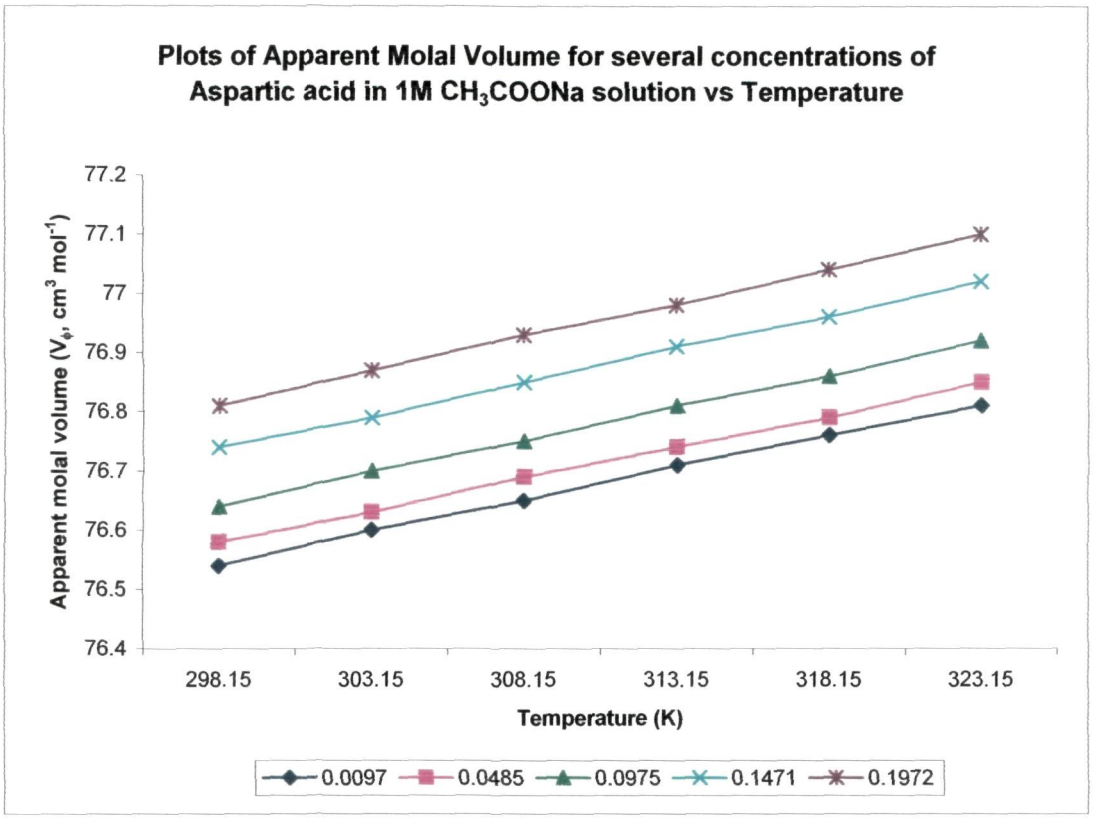


Fig. 1.5(a)

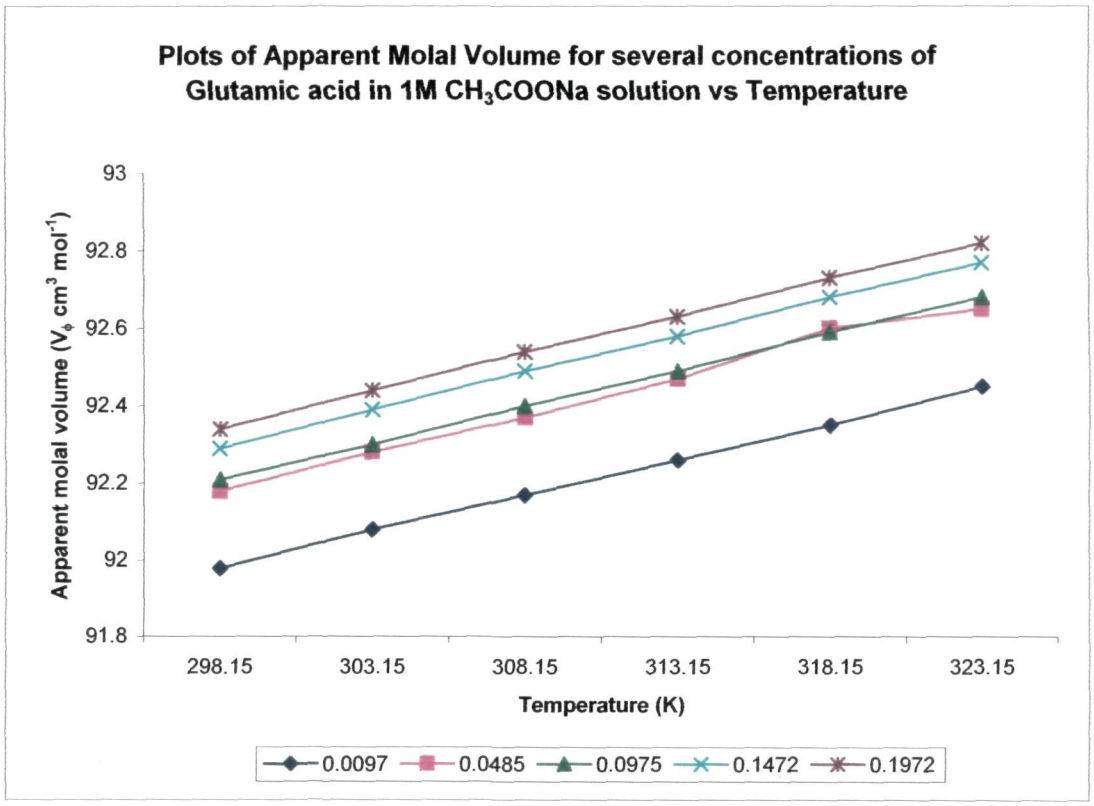


Fig. 1.5(b)

Table 1.6(c): Apparent Molal Volume V_ϕ (cm^3 mol^{-1}) of Aspartic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0094	0.0470	0.0944	0.1425	0.1910
298.15	77.88	77.93	78.03	78.11	78.15
303.15	77.92	77.97	78.07	78.15	78.19
308.15	77.97	78.01	78.12	78.19	78.24
313.15	78.01	78.05	78.16	78.24	78.28
318.15	78.05	78.09	78.20	78.28	78.32
323.15	78.10	78.14	78.25	78.33	78.37

Table 1.6(d): Apparent Molal Volume V_ϕ (cm^3 mol^{-1}) of Glutamic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0094	0.0467	0.0945	0.1426	0.1912
298.15	93.77	93.89	94.00	94.12	94.23
303.15	93.84	93.95	94.07	94.18	94.29
308.15	93.90	94.02	94.14	94.25	94.36
313.15	93.97	94.09	94.20	94.32	94.43
318.15	94.03	94.15	94.27	94.39	94.50
323.15	94.12	94.24	94.35	94.47	94.58

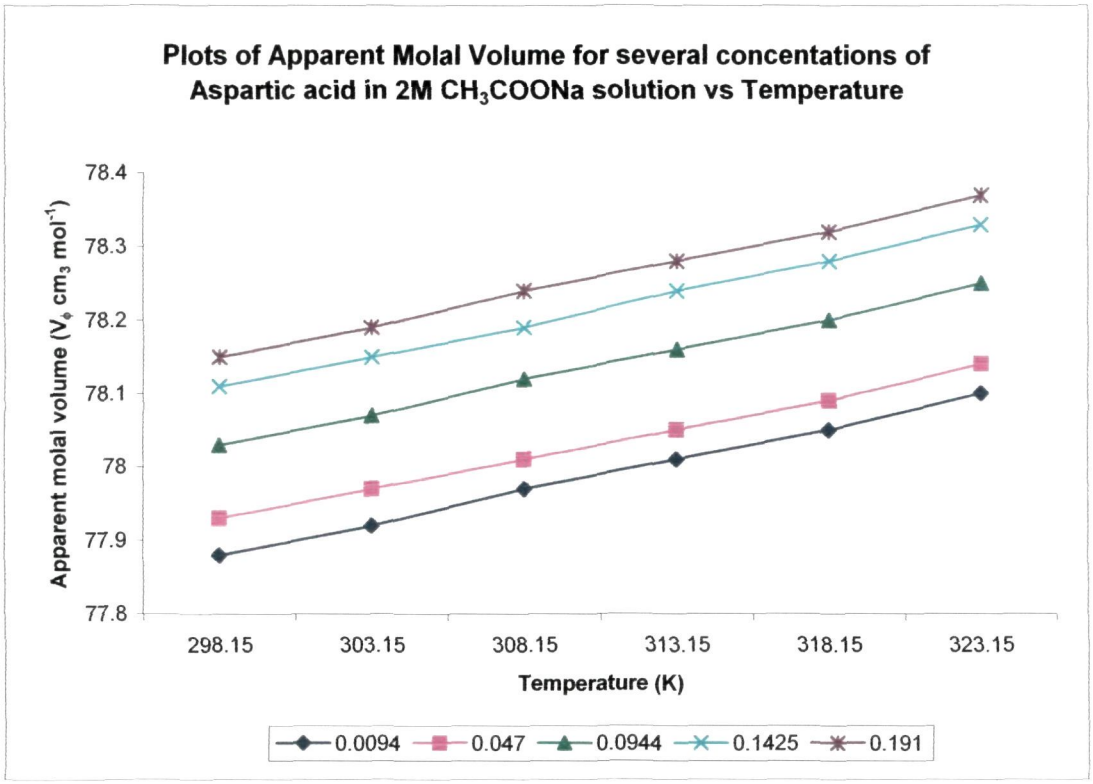


Fig. 1.5(c)

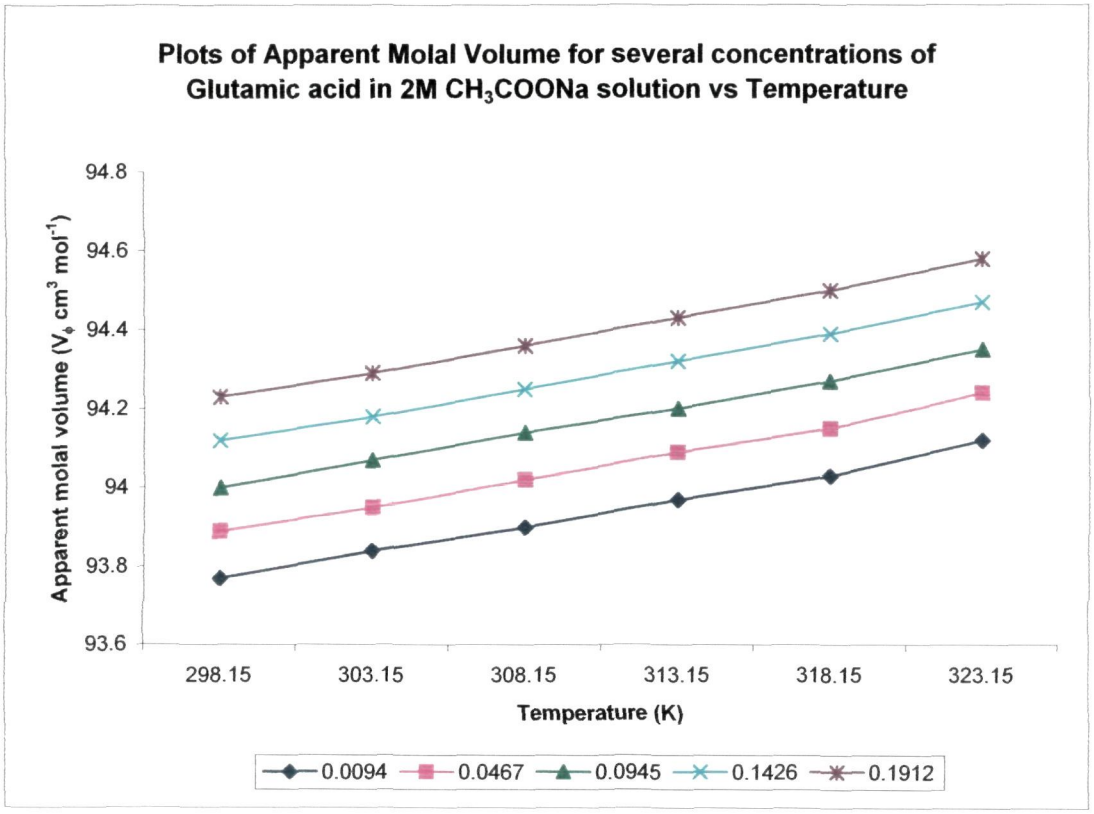


Fig. 1.5(d)

Table 1.6(e): Apparent Molal Volume V_ϕ (cm^3 mol^{-1}) of Aspartic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0096	0.0481	0.0969	0.1461	0.1959
298.15	77.76	77.90	77.97	78.06	78.11
303.15	77.82	77.96	78.03	78.12	78.17
308.15	77.89	78.03	78.10	78.19	78.24
313.15	77.95	78.09	78.16	78.26	78.31
318.15	78.01	78.16	78.23	78.32	78.37
323.15	78.08	78.22	78.29	78.38	78.44

Table 1.6(f): Apparent Molal Volume V_ϕ (cm^3 mol^{-1}) Glutamic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0096	0.0482	0.0965	0.1461	0.1959
298.15	93.11	93.32	93.44	93.56	93.64
303.15	93.21	93.42	93.54	93.66	93.74
308.15	93.32	93.53	93.65	93.77	93.85
313.15	93.42	93.63	93.75	93.87	93.96
318.15	93.52	93.74	93.85	93.97	94.06
323.15	93.62	93.84	93.96	94.08	94.17

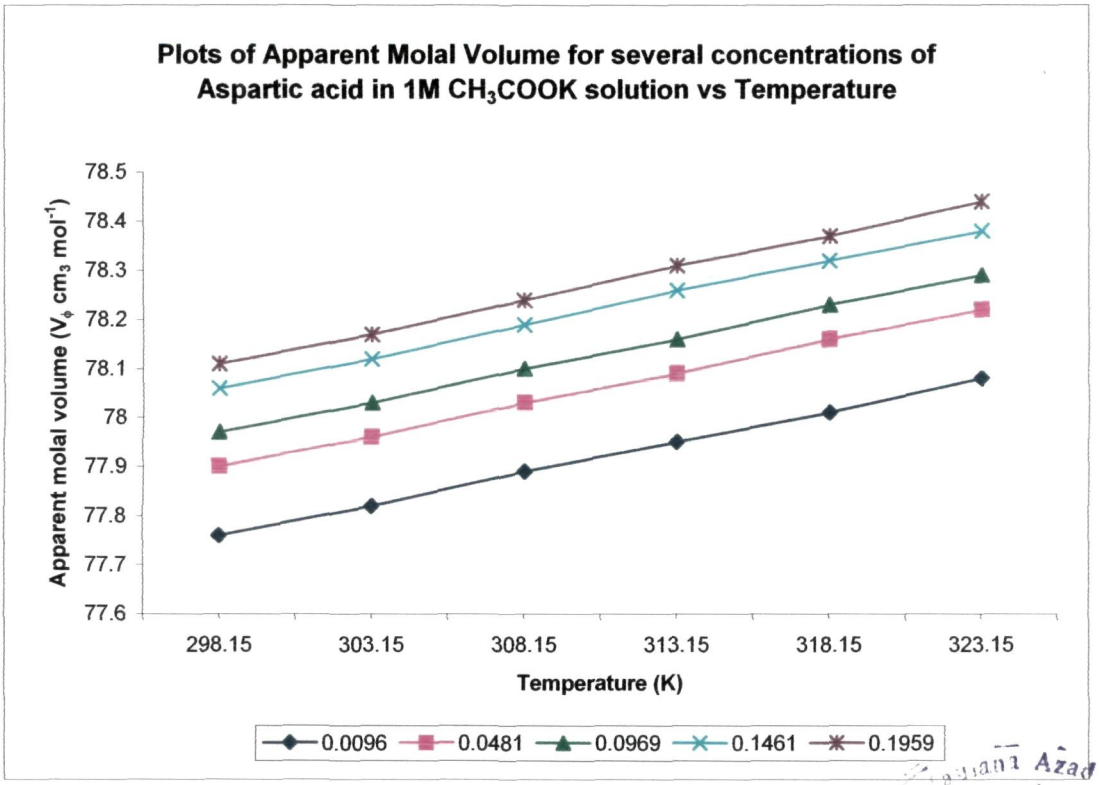


Fig. 1.5(e)

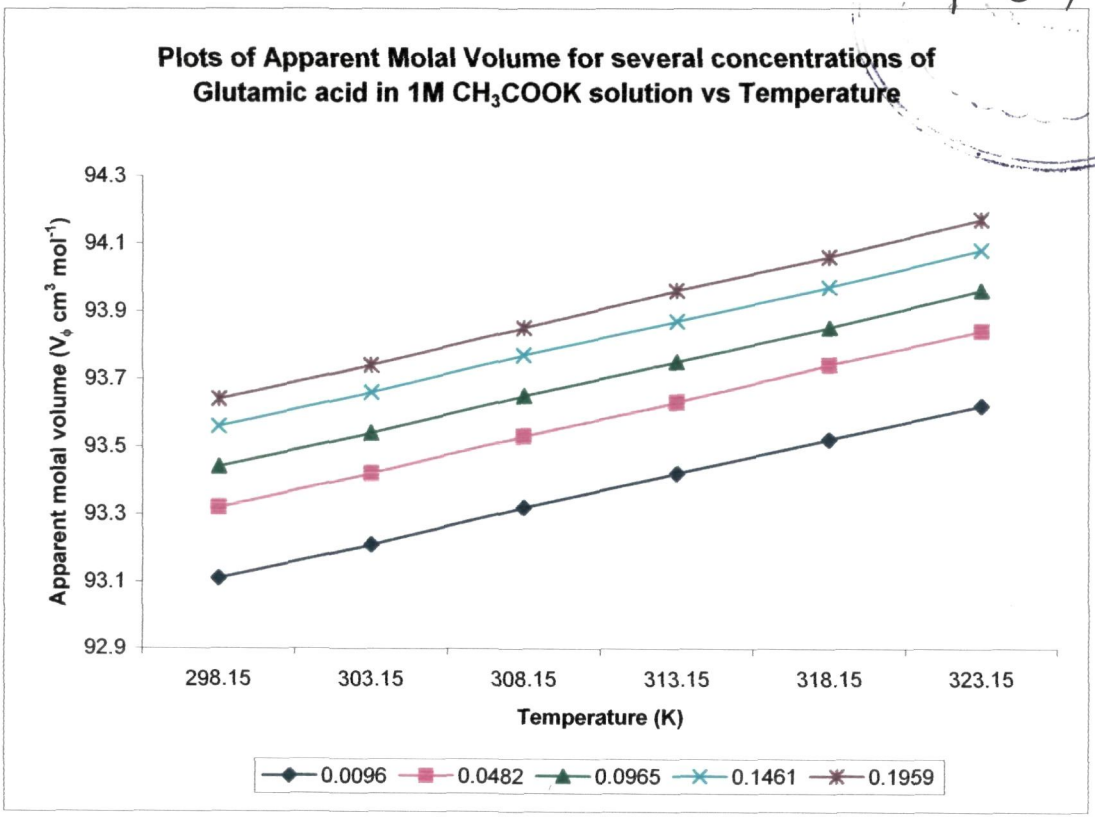


Fig. 1.5(f)

Table 1.7(a): Partial Molal Volume V_ϕ^0 (cm^3 mol^{-1}) of Aspartic acid in different co-solutes at various Temperatures.

Temp. K	1M CH_3COONa		2M CH_3COONa		1M CH_3COOK	
	V_ϕ^0	S_v	V_ϕ^0	S_v	V_ϕ^0	S_v
298.15	76.51	1.4838	77.87	1.5675	77.78	1.8093
303.15	76.57	1.4858	77.91	1.5675	77.84	1.8093
308.15	76.62	1.5270	77.95	1.5695	77.91	1.8093
313.15	76.68	1.5067	77.99	1.5911	77.97	1.8739
318.15	76.73	1.5500	78.03	1.5911	78.03	1.8497
323.15	76.78	1.5901	78.08	1.5911	78.10	1.8528

Table 1.7(b): Partial Molal Volume V_ϕ^0 (cm^3 mol^{-1}) of Glutamic acid in different co-solutes at various Temperatures.

Temp. K	1M CH_3COONa		2M CH_3COONa		1M CH_3COOK	
	V_ϕ^0	S_v	V_ϕ^0	S_v	V_ϕ^0	S_v
298.15	92.03	1.7221	93.76	2.4959	93.14	2.7342
303.15	92.13	1.7232	93.83	2.4534	93.24	2.7342
308.15	92.22	1.7863	93.89	2.4948	93.35	2.7342
313.15	92.31	1.7634	93.96	2.4959	93.45	2.7778
318.15	92.42	1.7348	94.02	2.5609	93.55	2.7548
323.15	92.50	1.7863	94.11	2.4959	93.65	2.8182

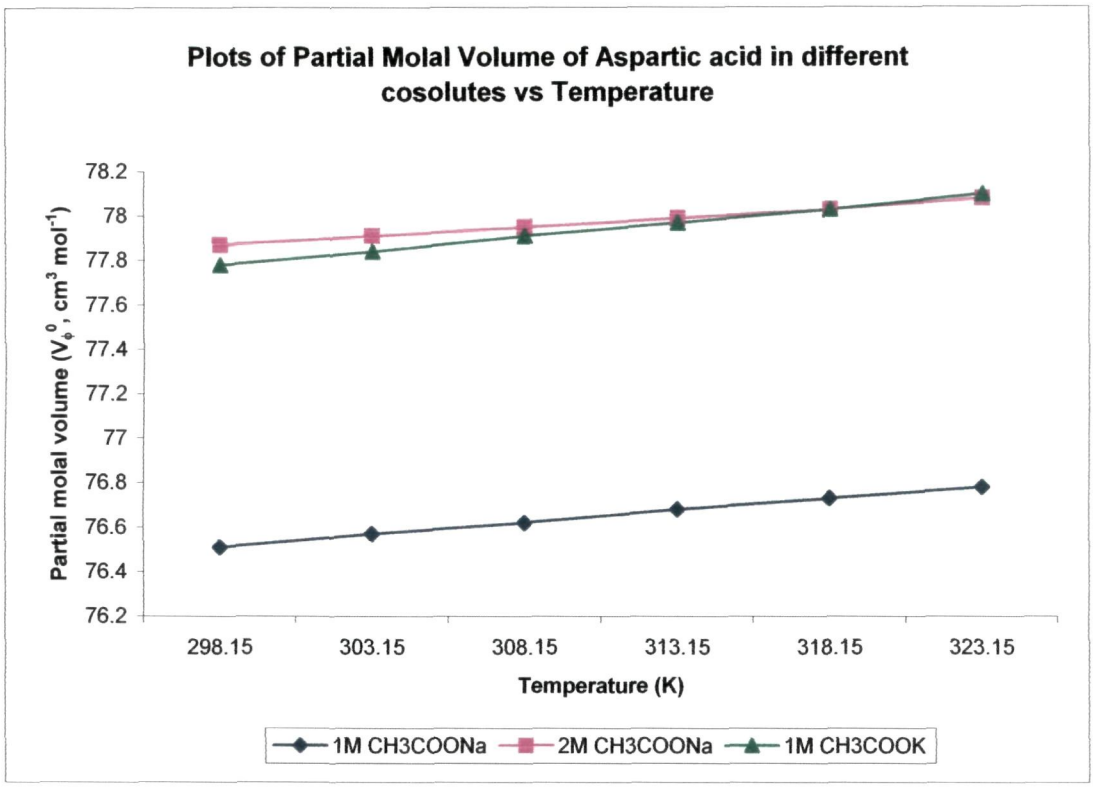


Fig. 1.6(a)

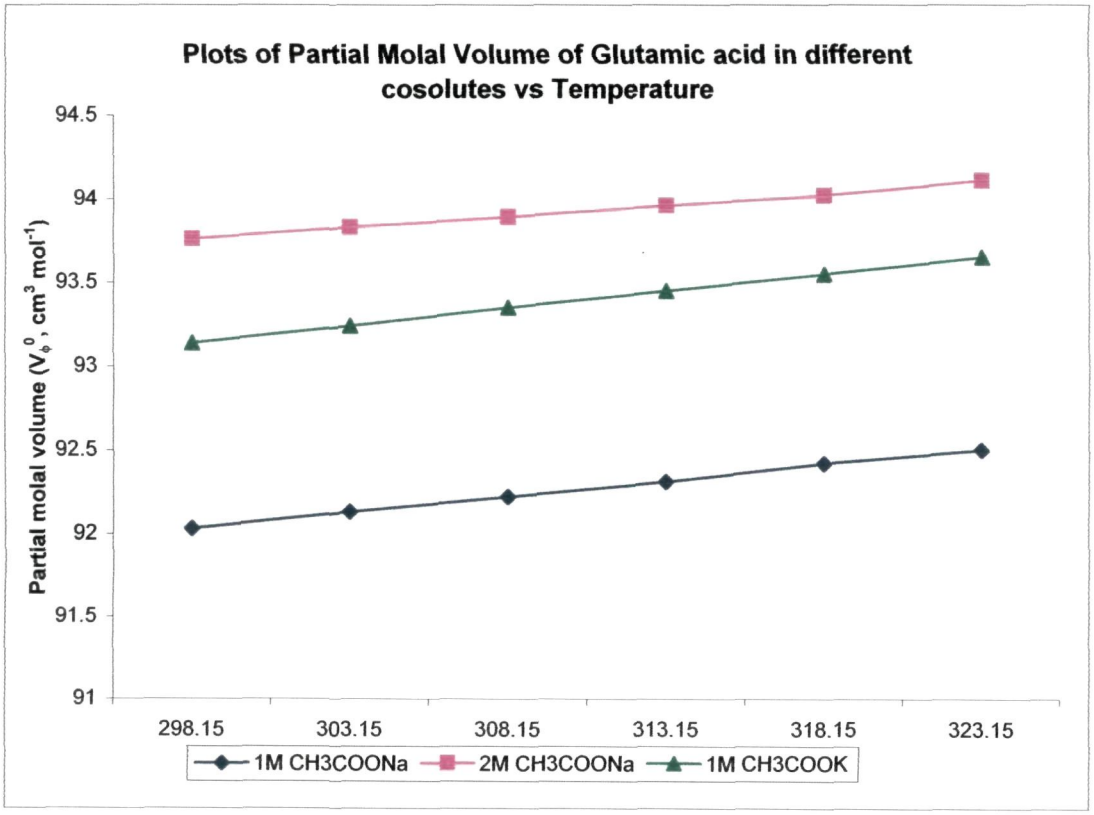


Fig. 1.6(b)

Table 1.7(c): Partial Molal Volume V_ϕ^0 (cm^3 mol^{-1}) of Aspartic acid and Glutamic acid in water at various Temperatures.

Temp. K	Aspartic acid	Glutamic acid
298.15	73.85	89.84
303.15	74.05	89.99
308.15	74.22	90.26
313.15	74.58	90.42
318.15	74.82	90.62
323.15	75.01	91.01

As seen from Tables 1.7 (a,b) the partial molal volume values of glutamic acid are higher than that for aspartic acid for all the concentrations studied. We propose an explanation for such behaviour which is as follows: For a short amino acid like aspartic acid, the distance between the two charged termini is very small, so the hydration shells of NH_3^+ and COO^- groups overlap and we could reasonably assume that the overall hydration of this amino acid is determined mostly by the electrostatic solute-solvent interaction, and any water molecules to which the CH_2 groups are accessible are predominantly under the influence of charged termini, thereby minimising the impact of CH_2 groups on hydration while the contribution of methylene group hydrophobic hydration to the partial molal volume is negligible.

In glutamic acid, which contains one more CH_2 group than aspartic acid, the separation between the oppositely charged amino and carboxyl terminal groups increases, so the interaction between these groups via overlapping of hydration shells decreases considerably and does not cause a measurable effect on the volume. Therefore, CH_2 groups become accessible to solvent molecule, which are not under the influence of the charged termini, and exerts an independent influence on the characteristics of adjacent water molecules, thus causing an increase in the partial molal volume. Present data [Tables 1.7(a,b)] at different temperatures are found to be consistent with the observations indicating the temperature independence of these qualitative features of dipolar hydration. However, by comparison the V_ϕ^0 data at different temperatures show a slight increase with the

increase of temperature, i.e., the values of $\partial V_\phi^0 / \partial T$ are positive [32-31]. Such an increase at higher temperatures is generally attributed to the increase in hydration volumes.

The partial molal volume of a solute at infinite dilution reflects the effect of solute-solvent interactions, while the magnitude of the slope is related to the solute-solute interactions. As can be seen from Tables 1.7(a,b), the values of the slope are positive, suggesting solute-solute interactions in the system. The slope increases with increase in temperature. This reflects the increased solute-solute interactions, which is probably due to the structure making effect of sodium or potassium acetate on addition of aspartic acid or glutamic acid on increasing temperature.

Tables 1.7(a,b) show that the partial molal volume (V_ϕ^0) values are positive and increase with increasing sodium acetate content in solution as well as with increasing temperature. This suggests the strong solute-solvent interactions in the mixtures.

It appears from Figs. 1.7(a,b) and Tables 1.8 (a,b) that the transfer volume values ($V_{\phi tr}^0$) are positive and increase linearly with increase in the concentration of sodium acetate. The magnitude of $V_{\phi tr}^0$ for aspartic acid is greater than that for glutamic acid, indicating that the contribution of CH_2 group to $V_{\phi tr}^0$ is negative and that of the zwitterionic group is positive.

The largest magnitudes of transfer volumes in case when CH_3COOK is cosolute than when it is CH_3COONa indicate that the aspartic acid or glutamic acid-CH_3COOK interactions are

Table 1.8(a): Partial Molal Volume of Transfer, $V_{\phi tr}^{0}$(cm^3 mol^{-1}) of Aspartic acid from water to different co-solutes at various Temperatures.

Temp. K	1M CH_3COONa	2M CH_3COONa	1M CH_3COOK
298.15	2.66	4.02	3.93
303.15	2.52	3.86	3.79
308.15	2.40	3.73	3.69
313.15	2.10	3.41	3.39
318.15	1.91	3.21	3.21
323.15	1.77	3.07	3.09

Table 1.8(b): Partial Molal Volume of Transfer, $V_{\phi tr}^{0}$(cm^3 mol^{-1}) of Glutamic acid from water to different co-solutes at various Temperatures.

Temp. K	1M CH_3COOKa	2M CH_3COONa	1M CH_3COOK
298.15	2.19	3.92	3.30
303.15	2.14	3.84	3.25
308.15	1.96	3.63	3.09
313.15	1.89	3.54	3.03
318.15	1.80	3.40	2.93
323.15	1.49	3.10	2.64

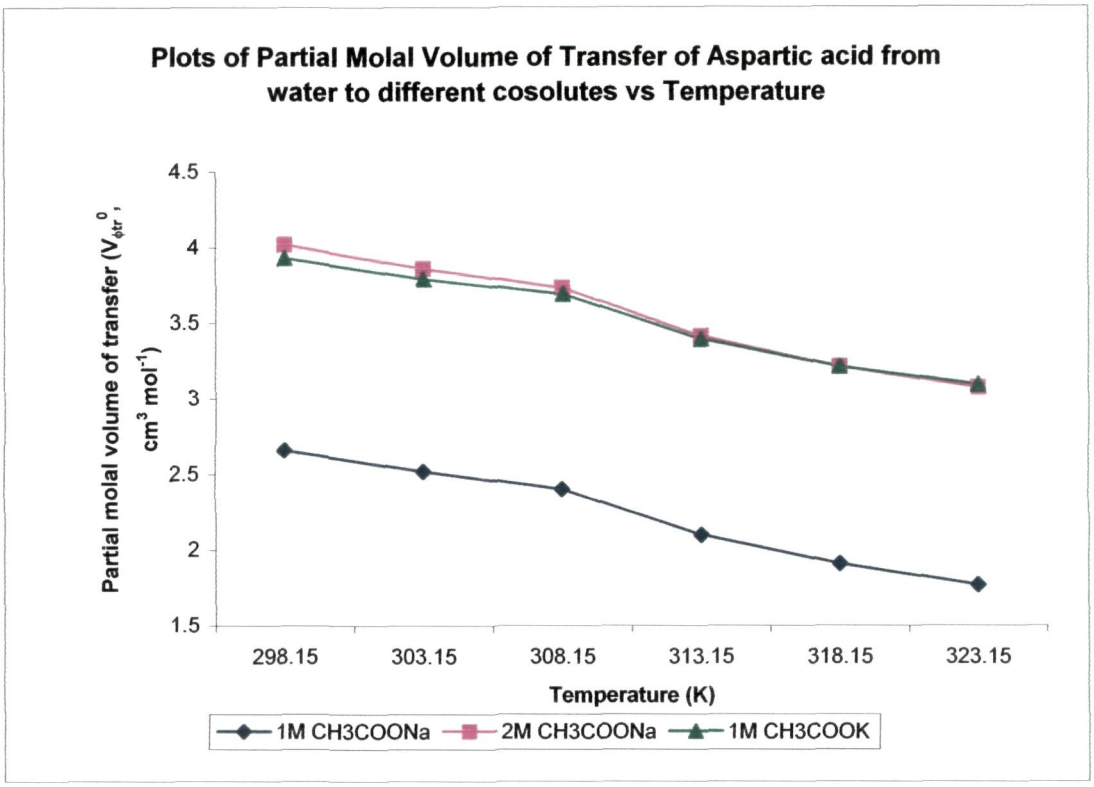


Fig. 1.7(a)

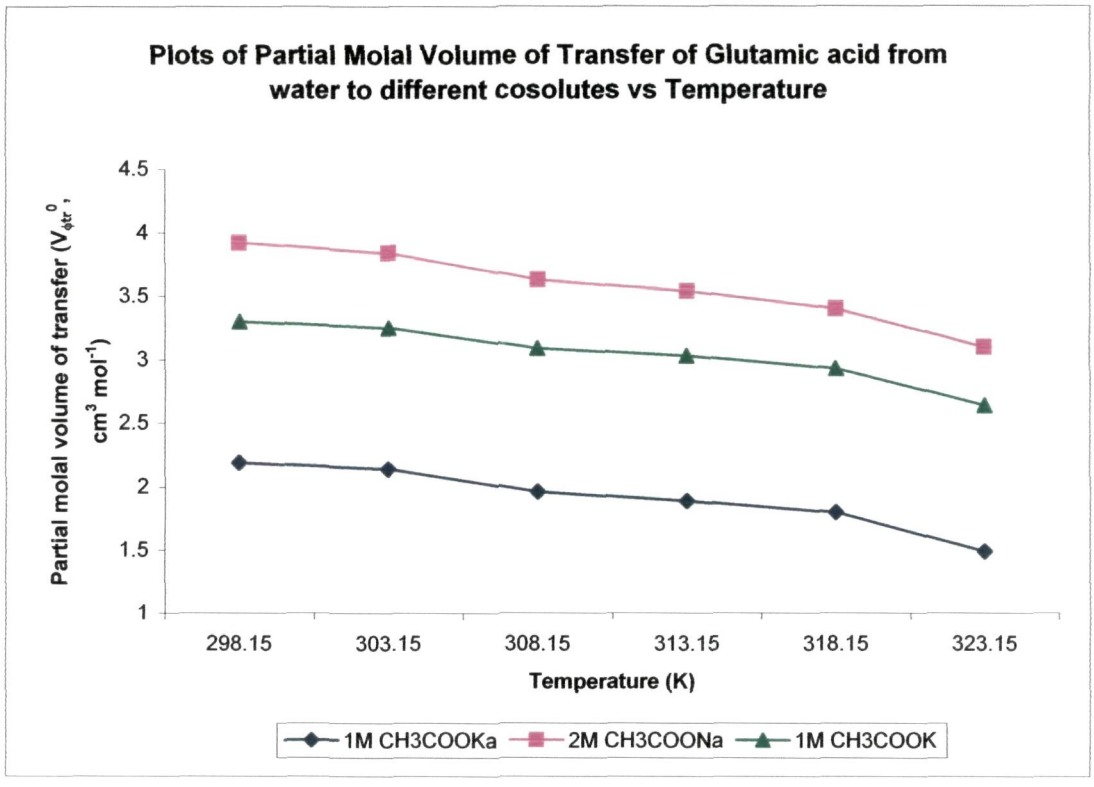


Fig. 1.7(b)

stronger than aspartic acid or glutamic acid-CH_3COONa interactions.

At neutral pH, amino acids exist as zwitterions and on dissolution in water there is an overall decrease in the volume of water. This is due to the contraction of the water near the end groups, and is termed as electrostriction. According to the Kirkwood model, addition of sodium or potassium acetate will coordinate the hydration spheres of the sodium or potassium ions with those of the carboxylate ions and acetate ions with the hydration sphere of the ammonium ion. As a result of these interactions, the water molecules are allowed to relax to the bulk state and this accounts for the positive transfer volumes. This is a qualitative interpretation of the results.

Franks et al. [34] have shown that the partial molal volume of a non-electrolyte is a combination of the intrinsic volume and the volume change due to its interaction with the solvent. The intrinsic volume has been considered to be made up of two types of contributions,

$$V_{\text{int}} = V_{vw} + V_{void}$$

where V_{vw} is the van der Walls' volume [35] and V_{void} is the volume associated with voids and empty spaces present therein [36]. Shahidi et. al. [37] modified this equation to include the contribution of interaction of a non-electrolyte solute with the solvent,

$$V_\phi^0 = V_{vw} + V_{void} - n\sigma_s$$

in which σ_s is the shrinkage in volume produced by the interactions of hydrogen bonding groups present in the solute

with water molecules and n is the potential number of hydrogen bonding sites in the molecule. For electrolytes and zwitterionic solutes, the shrinkage is caused by electrostriction and finally can be evaluated using the relation,

$$V_\phi^0 = V_{vw} + V_{void} - V_{shrinkage}\,.$$

If it is assumed that V_{vw} and V_{void} are of the same magnitude in water and salt solutions then the positive or negative values of the volume of transfer for the amino acids can have contributions from an increase or decrease in the volume of shrinkage in the presence of cosolutes in aqueous solution. Addition of sodium or potassium acetate decreases the electrostriction and this also means that $V_{shrinkage}$ in the above equation decreases as the electrostricted water becomes more like bulk water. Hence V_ϕ^0 increases on addition of sodium or potassium acetate and the values of $V_{\phi tr}{}^0$ are positive. Increasing the concentration of sodium acetate further decreases $V_{shrinkage}$ and hence $V_{\phi tr}{}^0$ increases as given in Tables 1.8 (a, b). Increasing the temperature also reduces the electrostriction and hence V_ϕ^0 increases.

The values of $V_{\phi tr}{}^0$ can be further rationalized by cosphere overlap model developed by Gurney [38] and Franks and Evans [39]. The properties of water molecules in the hydration cosphere depend on the nature of the solute species [40,41]. According to this model when the solute molecules approach each other, their hydration cosphere overlap and some of the cosphere material is displaced resulting in a change in the thermodynamic parameters [42,43]. In the amino acid-sodium acetate or potassium acetate-water ternary system, the following interactions may be

occurring; (a) ion-charged group interactions between Na^+ or K^+ and the COO^- groups of the amino acids and between CH_3COO^- and the NH_3^+ group of the amino acids; (b) ion-non-polar group interactions between Na^+ or K^+, CH_3COO^- and the non-polar groups of the amino acids. According to the co-sphere overlap model [38, 39], the ion-charged group interactions would lead to a positive $V_{\phi tr}^0$, whereas ion-non-polar group interactions will result in a negative $V_{\phi tr}^0$. The overall $V_{\phi tr}^0$ values obtained experimentally consist of these two opposite contributions. Since positive $V_{\phi tr}^0$ values were observed for both the amino acids studied, we may conclude that the contribution of ion-charged group interactions to $V_{\phi tr}^0$ dominates that of ion-non-polar group interactions due to smaller size of non-polar hydrocarbon chain. This also explains the higher $V_{\phi tr}^0$ values observed for higher molalities of sodium acetate. Greater values of $V_{\phi tr}^0$ of transfer of aspartic acid and glutamic acid in the presence of CH_3COOK than in the case of CH_3COONa suggest greater magnitude of ion-charged group interaction between K^+ and COO^- group of amino acids than that between Na^+ and COO^- group. The transfer volume values, $V_{\phi tr}^0$ decrease with temperature [Table 1.8 (a, b)] probably due to decrease in ion-charged group interactions as the temperature increases.

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Chapter-2

Viscosity, its related parameters and thermodynamic properties of acidic amino acids in salt solutions

Viscometry is a convenient and reliable experimental method, which gives valuable information about some hydrodynamic properties of macromolecules in solution and is still widely used [1-6].

Viscosity and its derived parameters provide valuable information regarding the shape and size of these molecules [7]. Such measurements on the dipolar ions, particularly of the amino acids, have been carried out by a number of workers [8-12]. However, the temperature effect on the solution has not been extensively studied for amino acids in mixed aqueous solvents. The addition of salts/solvents to protein solutions is known to affect their structure and configuration.

Studies on viscosity of ionic solutions are of great help in characterizing the structural properties of solutions. Various types of interactions exist between the ions in solutions and of these ion-ion and ion-solvent interactions are of current interest in all branches of chemistry. These interactions help in understanding the nature of solute and the solvent, i.e., whether the solute modifies or distorts the structure of solvent.

Viscosity of liquids is highly dependent on temperature. Even for simple liquids, viscosity-temperature relationships are quite complex. This has been evidenced by the large number of empirical expressions for this dependence, which have appeared in the literature [13,14]. For glass forming liquids, the William-Landel -Ferry equation [15] is widely used, which turns out to be applicable in the range from glass transition temperature Tg to about Tg+100°C. At higher temperatures, the temperature

dependence of viscosity is usually analyzed according to an equation of the Arrhenius form:

$$\eta = A\exp\left(\frac{\Delta E}{RT}\right) \qquad \text{(i)}$$

where η, ΔE, R and T are viscosity, activation energy of viscous flow, gas constant and absolute temperature, respectively. The pre-exponential factor A is considered to be independent or approximately independent of temperature. Equation (i) is still widely used for different liquids. [16-19].

Corradini et al. [20] and Palepu [21] have calculated the various thermodynamic parameters of activation of viscous flow by least squares fitting the density and the viscosity data to empirical equations stating their dependence on temperature and composition of the mixture. These parameters suggest the type and strength of interactions between the components of mixture. Palepu et al. [21] have calculated such thermodynamic parameters for the binary acid-base mixtures, while Corradini [20] and coworkers have obtained these for the binary mixtures of alcohols and amides.

Here we have calculated the viscosity and its related parameters (B-coefficient etc) and thermodynamic properties of aspartic and glutamic acid in different salt solutions (1M and 2M CH_3COONa and 1M CH_3COOK) at various temperatures.

Theory

When macromolecular material is added to a liquid, its viscosity is increased. Let us consider that the viscosity of a solvent is η_0. On addition of solute to the solvent, the viscosity of

the solvent increases to a new value η. The ratio of solution to solvent viscosity (η/η_0) is the relative viscosity,

$$\eta_{rel} = \frac{\eta}{\eta_0}. \qquad \text{(ii)}$$

The change in viscosity is generally expressed in terms of specific viscosity [22],

$$\eta_{sp} = \frac{\eta}{\eta_0} - 1. \qquad \text{(iii)}$$

As concentration of solute in the solution increases, the specific viscosity also increases. The quantity, η_{sp} in the limit of infinite dilution, is proportional to the concentration, measured in grams per milliliter. Thus, the quantity, $\frac{\eta_{sp}}{c}$ called the reduced viscosity must be independent at zero concentration.

Viscosity data can also be presented in a different way. One of the methods of the experimental results presentation, for different polymer systems, consists of using reduced variables. In the case of the viscosity-concentration dependence, this parameter is a dimensionless quantity [η]c, where [η] is the intrinsic viscosity and c is solute concentration. The intrinsic viscosity is given as

$$[\eta] = \lim_{C \to 0} \frac{\eta_{sp}}{c}. \qquad \text{(iv)}$$

The principal method of determination of the magnitude of intrinsic viscosity consists of plotting the η_{sp}/c against

concentration and extrapolating it to the intercept, which is equal to [η].

We have calculated the intrinsic viscosities by least-squares fitting of the data [23] to the relevant expression (v). The temperature and concentration effects on the kinematic viscosity have been investigated. The intrinsic viscosities [η] were computed by the least-squares method, using the following equation:

$$\eta_{red} = [\eta] + K[\eta]^2 c \qquad \text{(v)}$$

where K is Huggin's constant.

The relative viscosity, η_r can be represented by the relation [24,10,11],

$$\eta_r = \frac{\eta}{\eta_0} = 1 + Bc\,. \qquad \text{(vi)}$$

The B-coefficient values of the solute are obtained by the least-squares procedure. B-coefficient is the measure of order or disorder introduced by the solute into solvent structure. This constant is specific and is an approximately additive property of ions of an electrolyte at a given temperature, although no satisfactory theoretical treatment has yet been given.

Viscosity data have also been used for the calculation of solute activation parameters [25]. The free energy of activation for viscous flow is given by Eyring viscosity equation [28],

$$\eta = \frac{hN}{V_m} e^{(\Delta G^*/RT)} \qquad \text{(vii)}$$

in which h is the Planck's constant, N is Avagadro's number, R is the universal gas constant and V_m is the molar volume of the mixture. Molar volume of the mixture has been calculated from the corresponding mixture densities by the following relation:

$$V_m = \sum_i \frac{X_i M_i}{\rho}, \qquad i = 1,2,3,... \qquad \text{(viii)}$$

The energies of activation ΔG* for viscous flow of the solute at different temperatures are obtained by using equation (ix),

$$\Delta H^* = \Delta G^* + T\Delta S^* \qquad \text{(ix)}$$

where ΔH* and ΔS* are the enthalpy and entropy of activation for the viscous flow of solute. From equations (vii) and (ix), we get,

$$\Delta G^* = RT \ln \frac{\eta V_m}{hN} = \Delta H^* - T\Delta S^*. \qquad \text{(x)}$$

The values of ΔH* and ΔS* can be obtained by least-squares fitting. ΔS* is the corresponding experimental slope of $RT \ln \frac{\eta V_m}{hN}$ vs temperature plots.

Results and Discussion

The viscosities of aspartic and glutamic acids in different salt solutions are given in Tables 2.1(a-f)) for several molalities of solutes at different temperatures. As seen in the above tables, viscosity η decreases with the increase in temperature. It is attributed to the fact that as the temperature is increased the solution becomes more active. This increased molecular activity or molecular motion occurs at the expense of cohesive forces acting between the molecules. As a result, the liquid now faces lesser

Table 2.1(a) Viscosity ($\eta \times 10^4$, kg $m^{-1}s^{-1}$) of Aspartic acid in 1M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0097	0.0485	0.0975	0.1471	0.1972
298.15	1.2384	1.2422	1.2605	1.2749	1.2924	1.3116
303.15	1.0808	1.0922	1.1038	1.1113	1.1298	1.1436
308.15	0.9803	0.9839	0.9906	0.9978	1.0113	1.0248
313.15	0.8888`	0.8939	0.9059	0.9168	0.9270	0.9388
318.15	0.7952	0.8002	0.8126	0.8286	0.8354	0.8469
323.15	0.7253	0.7303	0.7364	0.7490	0.7708	0.7835

Table 2.1(b): Viscosity ($\eta \times 10^4$, kg $m^{-1}s^{-1}$) of Glutamic acid in 1M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0097	0.0485	0.0975	0.1472	0.1972
298.15	1.2384	1.2815	1.2935	1.3124	1.3268	1.3570
303.15	1.0808	1.1015	1.1254	1.1437	1.1683	1.1759
308.15	0.9803	0.9916	1.0013	1.0115	1.0421	1.0618
313.15	0.8888	0.9016	0.9174	0.9275	0.9423	0.9619
318.15	0.7952	0.8186	0.8310	0.8439	0.8584	0.8621
323.15	0.7253	0.7439	0.7561	0.7625	0.7736	0.7847

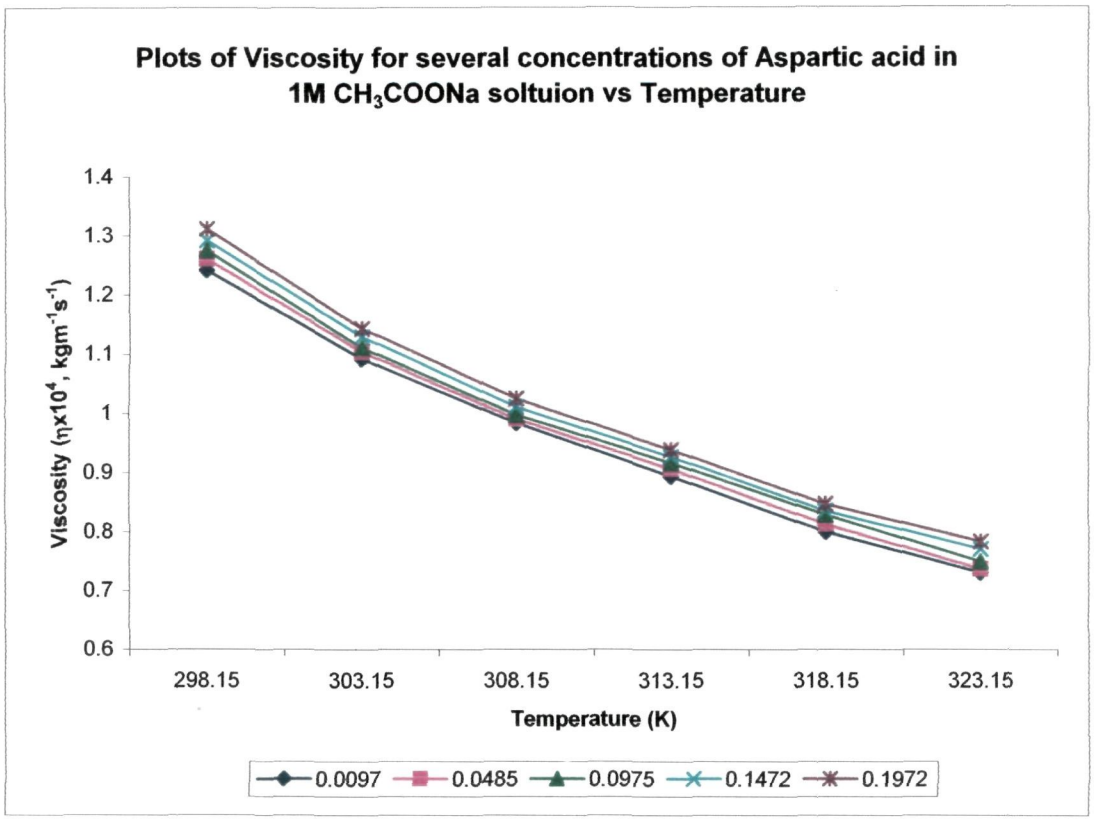

Fig. 2.1(a)

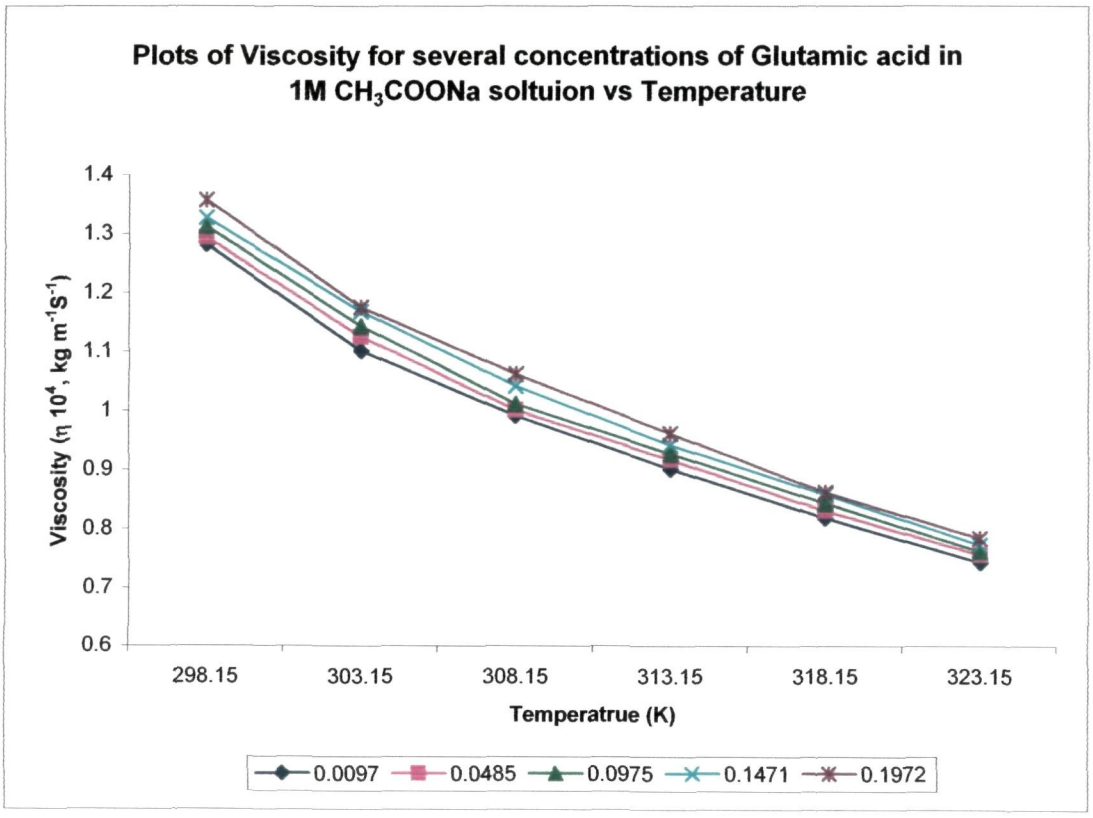

Fig. 2.1(b)

Table 2.1(c): Viscosity ($\eta \times 10^4$, kg $m^{-1}s^{-1}$) of Aspartic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0094	0.0470	0.0944	0.1425	0.1910
298.15	1.6834	1.6874	1.7652	1.7823	1.7987	1.8184
303.15	1.4697	1.4752	1.5436	1.5584	1.5733	1.5898
308.15	1.2915	1.2969	1.3809	1.3905	1.3986	1.4034
313.15	1.1864	1.1982	1.2423	1.2516	1.2651	1.2785
318.15	1.0496	1.0564	1.0746	1.0928	1.1274	1.1509
323.15	0.9516	0.9552	0.9964	1.0098	1.0177	1.0288

Table 2.1(d): Viscosity ($\eta \times 10^4$, kg $m^{-1}s^{-1}$) of Glutamic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0094	0.0467	0.0945	0.1426	0.1912
298.15	1.6834	1.7246	1.7552	1.7965	1.8313	1.8679
303.15	1.4697	1.5087	1.5305	1.5563	1.5870	1.6074
308.15	1.2915	1.3447	1.3695	1.4013	1.4108	1.4372
313.15	1.1864	1.2078	1.2292	1.2609	1.2685	1.2891
318.15	1.0496	1.0644	1.1045	1.1244	1.1365	1.1582
323.15	0.9516	0.9725	0.9947	1.0095	1.0228	1.0345

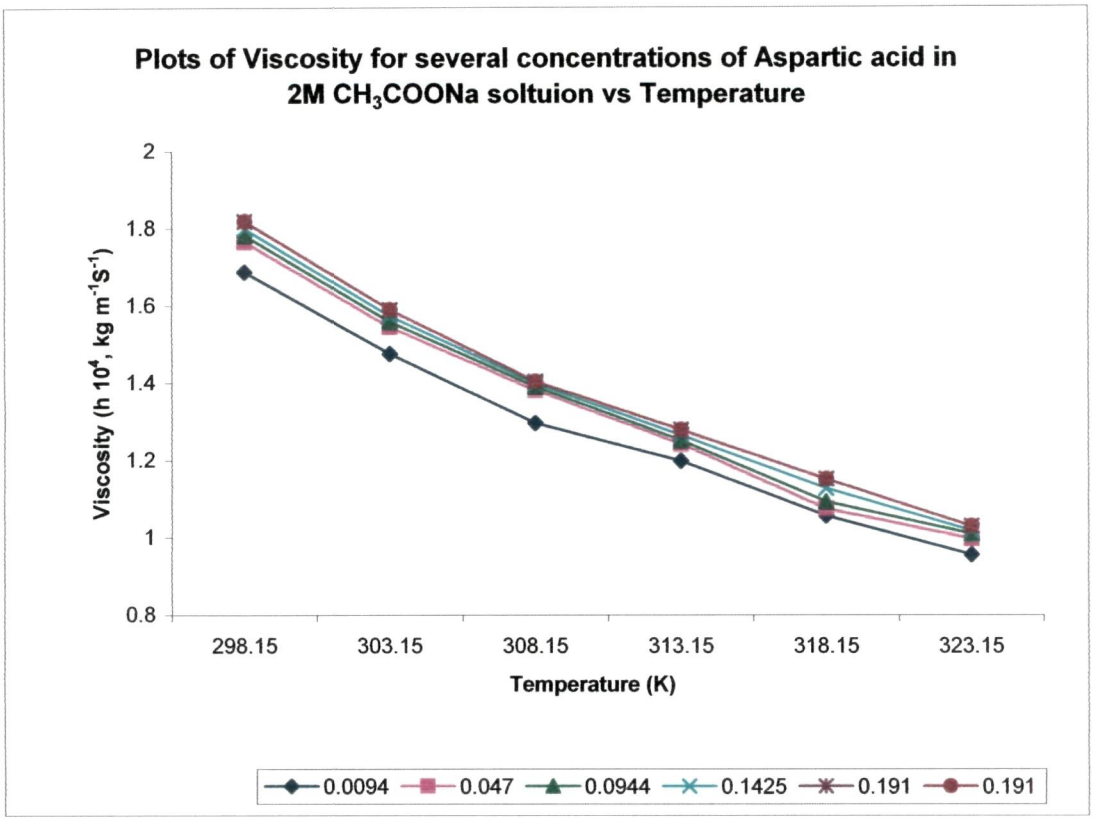

Fig. 2.1(c)

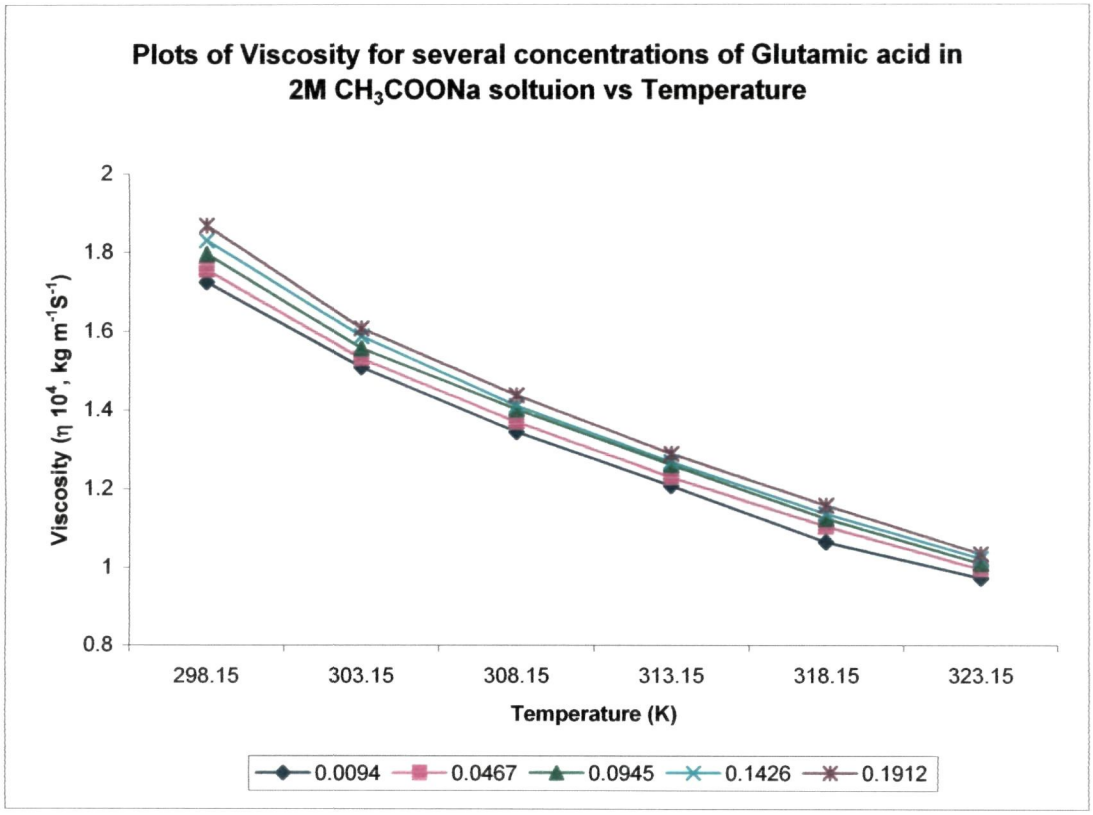

Fig. 2.1(d)

Table 2.1(e): Viscosity ($\eta \times 10^4$, kg $m^{-1}s^{-1}$) of Aspartic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0096	0.0481	0.0969	0.1461	0.1959
298.15	0.8862	1.1158	1.1497	1.1716	1.2047	1.2316
303.15	0.8077	0.9825	1.0016	1.0166	1.0505	1.0719
308.15	0.7301	0.8766	0.8970	0.9102	0.9422	0.9602
313.15	0.6805	0.8136	0.8184	0.8361	0.8743	0.8813
318.15	0.6225	0.7294	0.7464	0.7622	0.7812	0.7894
323.15	0.5665	0.6706	0.6766	0.6890	0.7107	0.7341

Table 2.1(f): Viscosity ($\eta \times 10^4$, kg $m^{-1}s^{-1}$) of Glutamic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0096	0.0482	0.0965	0.1461	0.1959
298.15	0.8862	1.1632	1.1828	1.2054	1.2202	1.2534
303.15	0.8077	0.9918	1.0233	1.0273	1.0595	1.0903
308.15	0.7301	0.8921	0.9047	0.9319	0.9482	0.9661
313.15	0.6805	0.8183	0.8261	0.8437	0.8678	0.8919
318.15	0.6225	0.7387	0.7479	0.7776	0.7841	0.8156
323.15	0.5665	0.6752	0.6811	0.7042	0.7167	0.7415

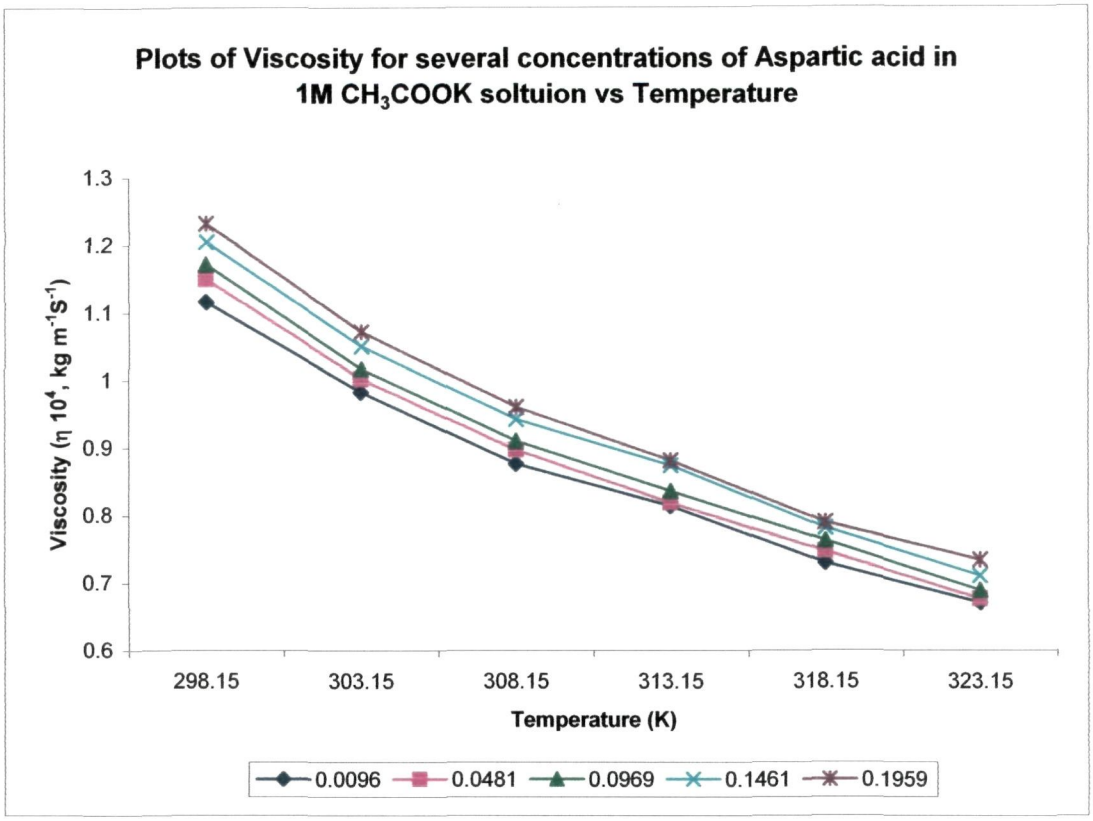

Fig. 2.1(e)

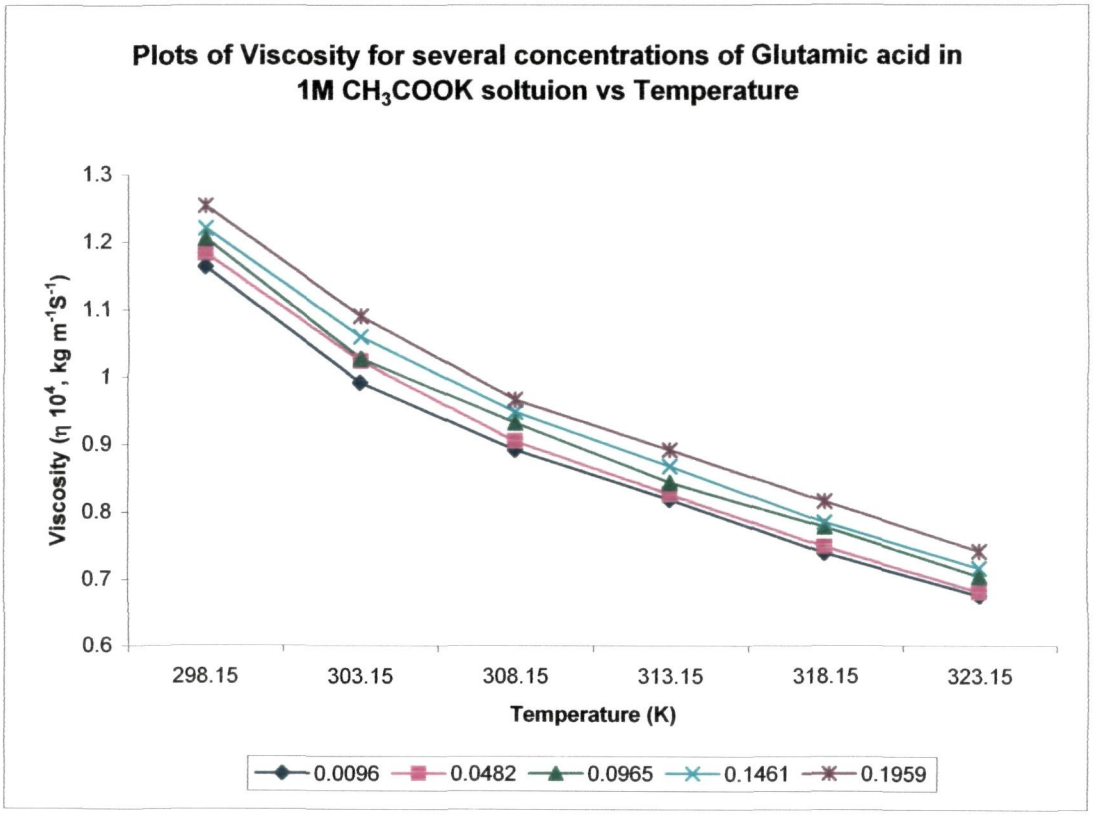

Fig. 2.1(f)

resistance to its flow and the liquid now flows easily or we can say the liquid has become more mobile.

It can be seen that as the temperature increases, the solvation sphere surrounding the ion slowly breaks down showing a decrease in the relative viscosity [Tables 2.2(a-f)].

The intrinsic viscosity measures the contribution of a protein to the viscosity of solution in which it is dissolved. On the other hand, it is a measure of the additional loss of energy caused by the rotational motion of dissolved macromolecules during a viscous flow, as shown in Tables 2.4 (a,b). [η] generally decreases with increasing temperature in all the systems under investigation.

It is well known that the Huggins coefficient represents a rheological measure of intermolecular interactions. Because, for different liquids and solutions these interactions can be different, in each case K has to be calculated separately. There is no regular pattern of K with increasing temperature [Tables 2.3(a,b)].

B-coefficient is calculated using equation (vi) and its values are given in Tables 2.4(a,b). It was found that in determining the viscosity of an amino acid, the charge distribution is less important than the size and structure of the hydrocarbon chain. Due to the large size and non-electrolytic nature, all the amino acids, irrespective of the structure, show significant positive core contribution to the B-coefficient, which exceeds any negative contribution. All the dipolar ions including amino acids will exhibit a positive B-coefficient.

Table 2.2(a): Relative Viscosity, η_r of Aspartic acid in 1M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1471	0.1972
298.15	1.0031	1.0178	1.0294	1.0436	1.0590
303.15	1.0105	1.0213	1.0282	1.0453	1.0581
308.15	1.0037	1.0105	1.0179	1.0316	1.0454
313.15	1.0058	1.0192	1.0315	1.0430	1.0563
318.15	1.0063	1.0220	1.0421	1.0507	1.0651
323.15	1.0068	1.0152	1.0326	1.0627	1.0803

Table 2.2(b): Relative Viscosity, η_r of Glutamic acid in 1M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1472	0.1972
298.15	1.0347	1.0444	1.0598	1.0713	1.0957
303.15	1.0191	1.0412	1.0582	1.0810	1.0880
308.15	1.0115	1.0215	1.0319	1.0630	1.0832
313.15	1.0145	1.0322	1.0436	1.0603	1.0823
318.15	1.0295	1.0451	1.0613	1.0795	1.0841
323.15	1.0256	1.0424	1.0513	1.0666	1.0819

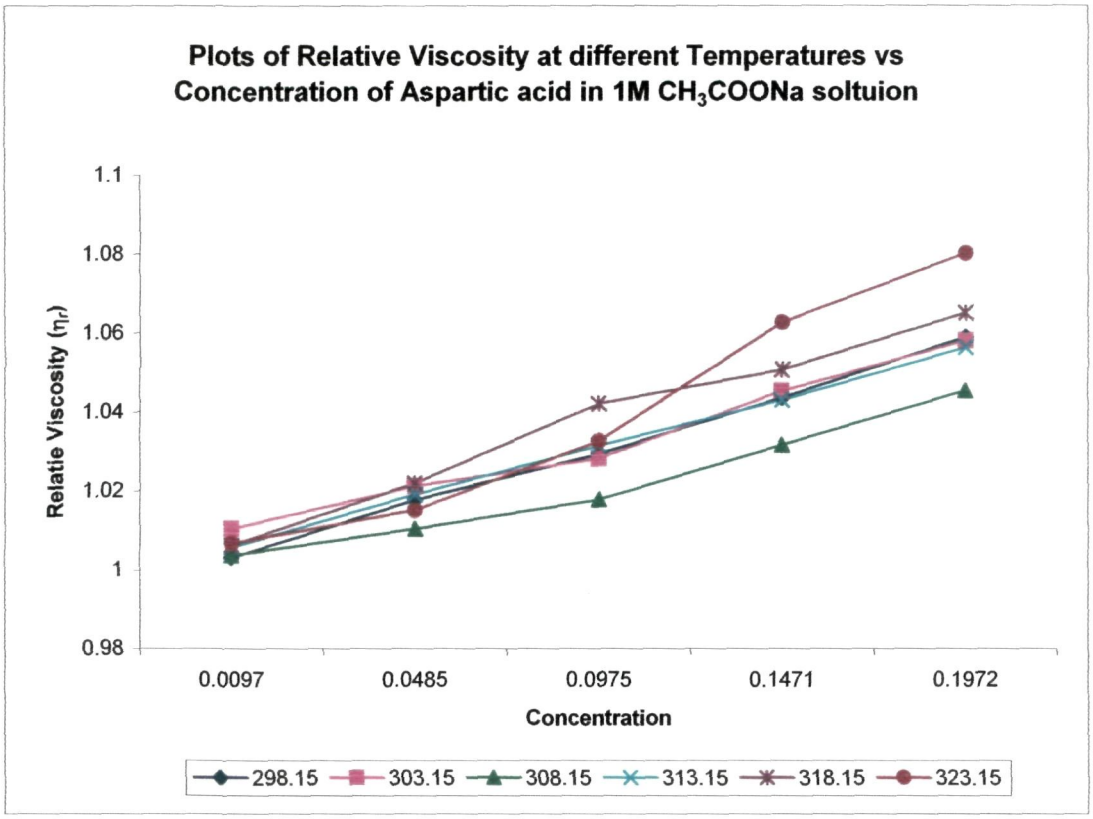

Fig. 2.2(a)

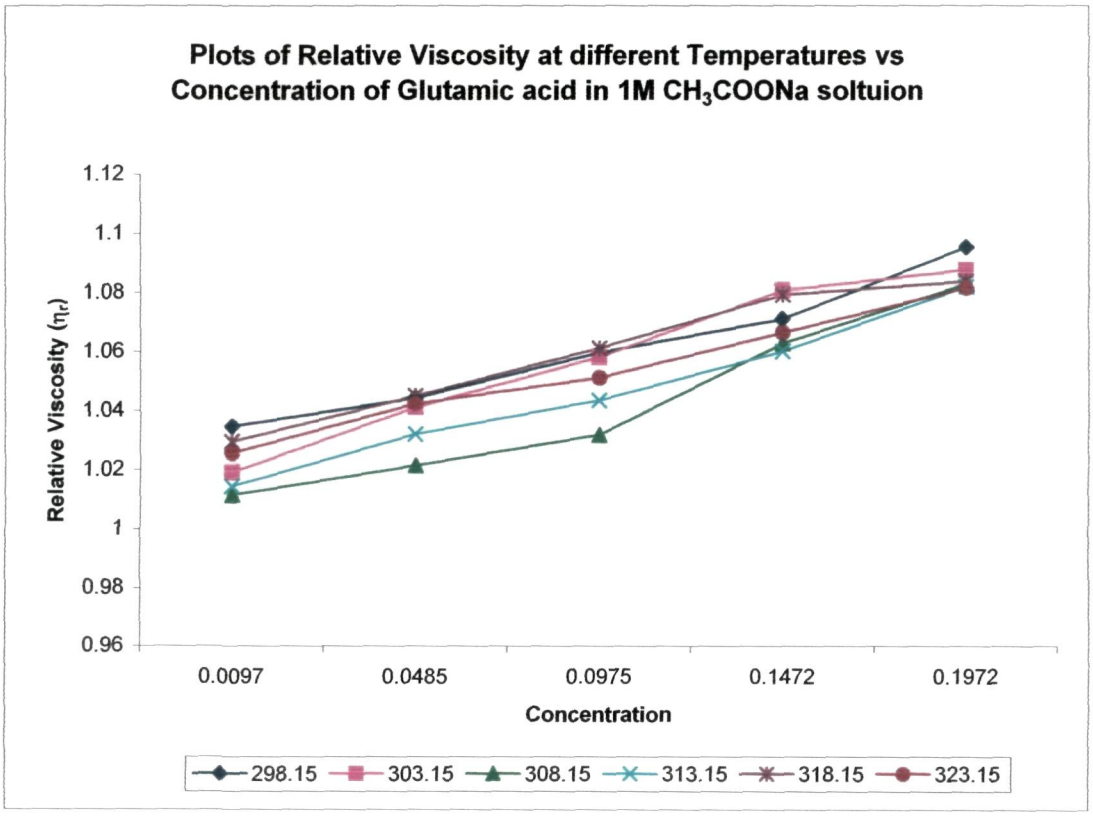

Fig. 2.2(b)

Table 2.2(c): Relative Viscosity, η_r of Aspartic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0094	0.0470	0.0944	0.1425	0.1910
298.15	1.0024	1.0486	1.0588	1.0685	1.0802
303.15	1.0037	1.0503	1.0603	1.0705	1.0817
308.15	1.0042	1.0692	1.0767	1.0829	1.0867
313.15	1.0049	1.0471	1.0550	1.0663	1.0776
318.15	1.0065	1.0238	1.0412	1.0741	1.0965
323.15	1.0038	1.0471	1.0611	1.0694	1.0811

Table 2.2(d): Relative Viscosity, η_r of Glutamic acid in 2M CH_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0094	0.0467	0.0945	0.1426	0.1912
298.15	1.0245	1.0427	1.0672	1.0879	1.1096
303.15	1.0265	1.0414	1.0590	1.0798	1.0937
308.15	1.0413	1.0604	1.0851	1.0924	1.1128
313.15	1.0180	1.03608	1.03608	1.0628	1.0628
318.15	1.0141	1.0523	1.0713	1.0828	1.1035
323.15	1.0219	1.0453	1.0608	1.0748	1.0871

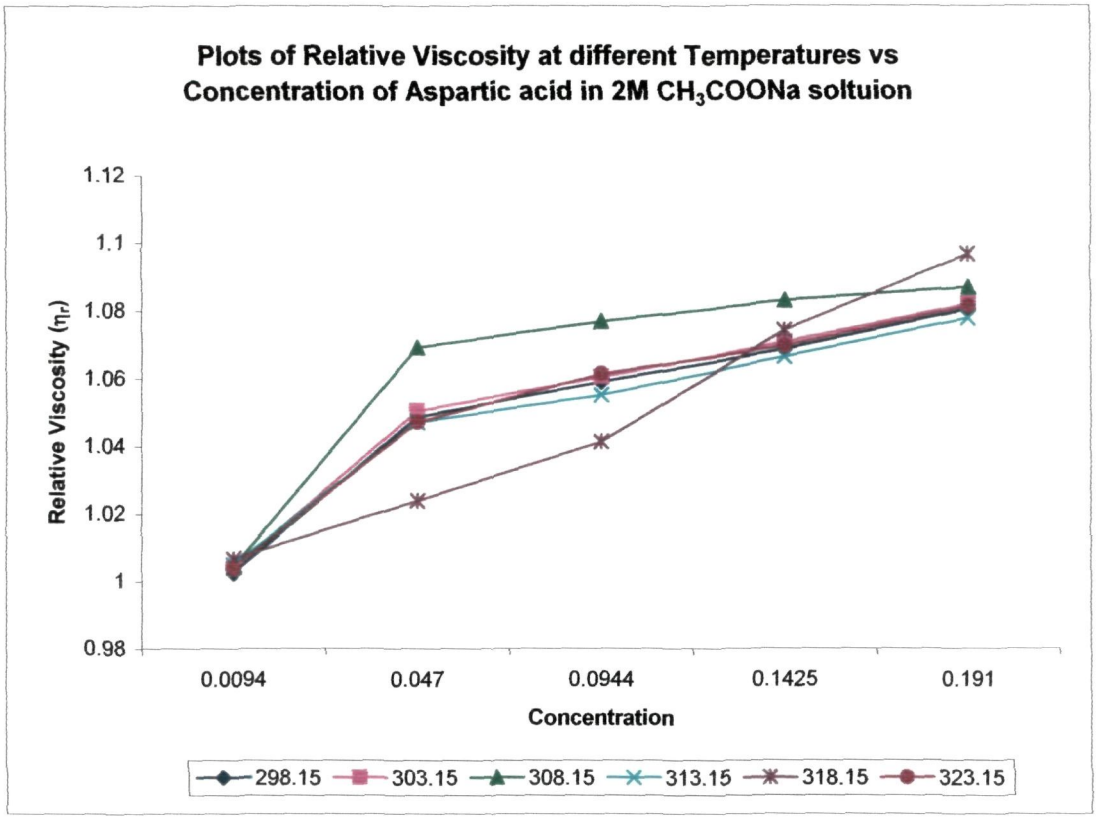

Fig. 2.2(c)

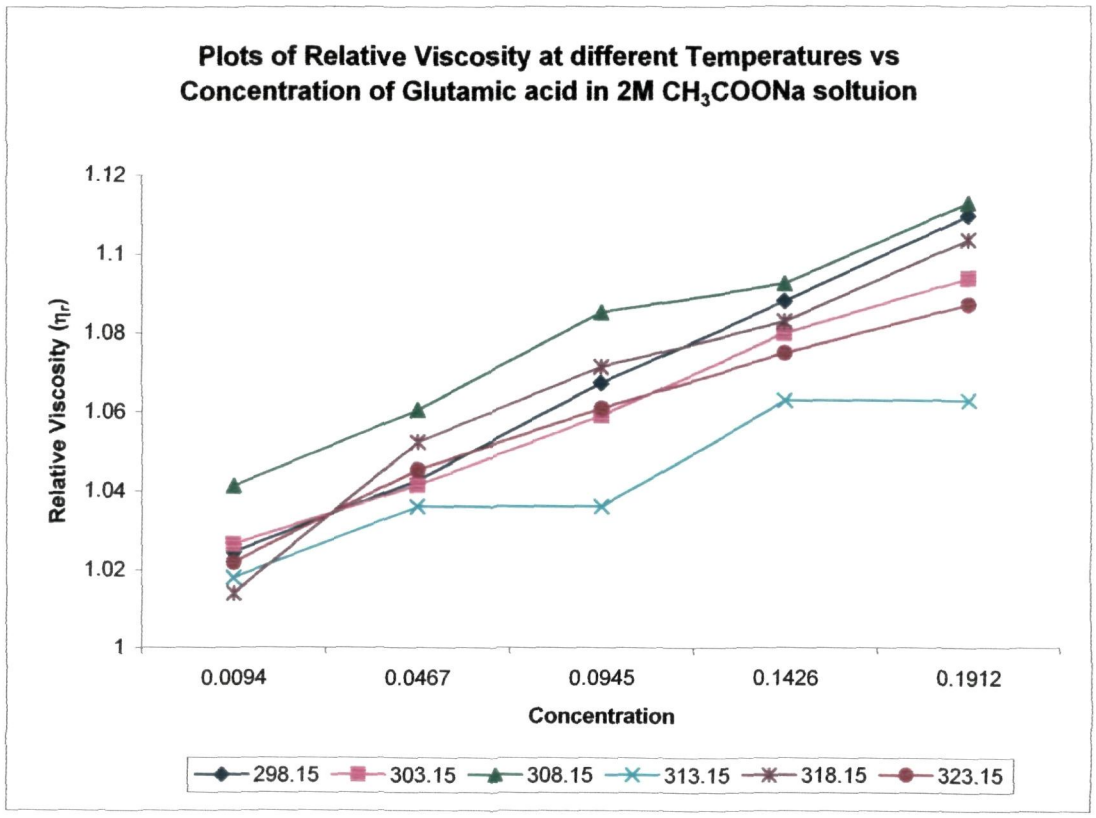

Fig. 2.2(d)

Table 2.2(e): Relative Viscosity, η_r of Aspartic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0096	0.0481	0.0969	0.1461	0.1959
298.15	1.2591	1.2973	1.3221	1.3594	1.3898
303.15	1.2164	1.2401	1.2587	1.3006	1.3272
308.15	1.2006	1.2286	1.2466	1.2904	1.3151
313.15	1.1956	1.2025	1.2286	1.2848	1.2950
318.15	1.1718	1.1990	1.2245	1.2550	1.2682
323.15	1.1839	1.1944	1.2163	1.2547	1.2959

Table 2.2(f): Relative Viscosity, η_r of Glutamic acid in 1M CH_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0096	0.0482	0.0965	0.1461	0.1959
298.15	1.3126	1.3347	1.3603	1.3769	1.4144
303.15	1.2280	1.2670	1.2719	1.3118	1.3499
308.15	1.2219	1.2391	1.2763	1.2986	1.3231
313.15	1.2024	1.2139	1.2398	1.2751	1.3106
318.15	1.1867	1.2014	1.2491	1.2597	1.3102
323.15	1.1919	1.2023	1.2431	1.2651	1.3089

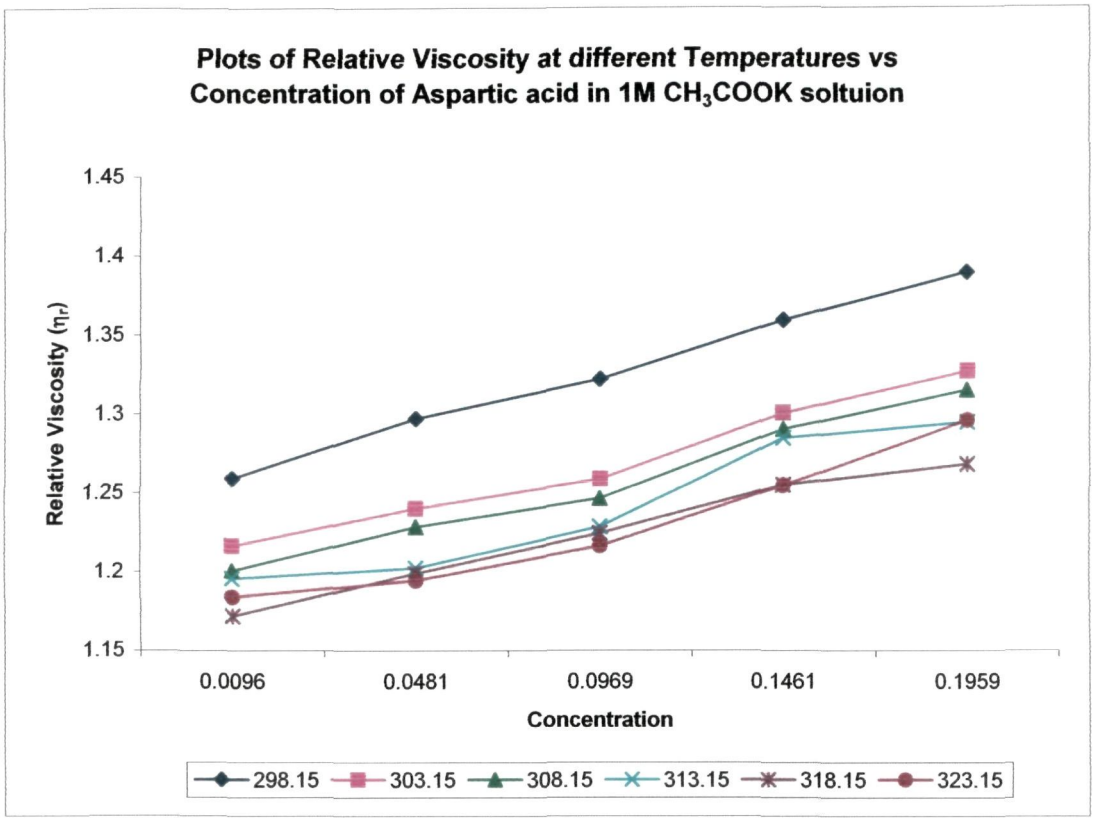

Fig. 2.2(e)

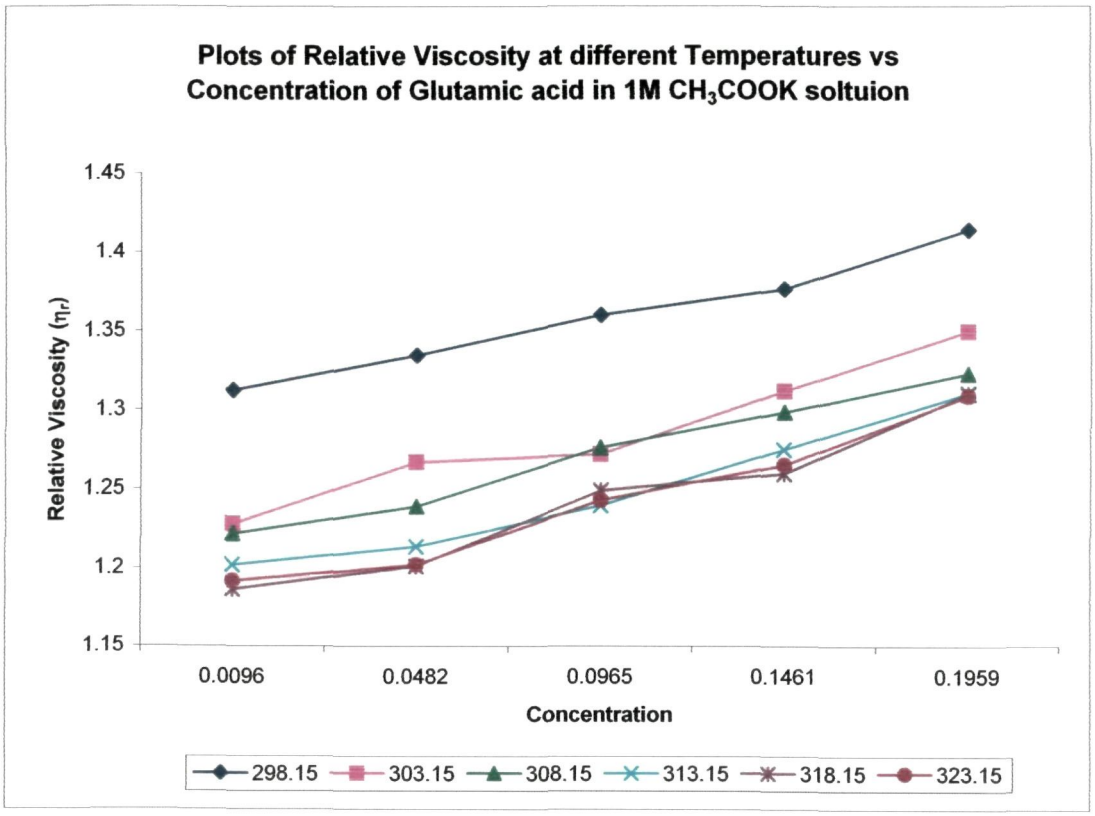

Fig. 2.2(f)

Table 2.3(a): The numerical values of the Intrinsic Viscosity ([η], m^3/kg) and Huggins Coefficient (K) for Aspartic acid in different salt solutions at different Temperatures.

Temp. K	[η]	K
In 1M CH_3COONa		
298.15	0.3302	-1.8903
303.15	0.8075	-5.0733
308.15	0.2966	-6.3827
313.15	0.5136	-5.3563
318.15	0.5729	-4.4300
323.15	0.5126	-3.2334
In 2M CH_3COONa		
298.15	0.5872	-1.5850
303.15	0.6818	-2.3270
308.15	0.9021	-2.2964
313.15	0.7212	-2.9925
318.15	0.5665	-1.9413
323.15	0.6629	-2.2893
In 1M CH_3COOK		
298.15	18.4616	-0.3043
303.15	15.3242	-0.3670
308.15	14.2575	-0.3924
313.15	13.7161	-0.4097
318.15	12.2677	-0.4546
323.15	12.9125	-0.4345

Table 2.3(b): The numerical values of the Intrinsic Viscosity ([η], m^3/kg) and Huggins Coefficient (K) for Glutamic acid in different salt solutions at different Temperatures.

Temp. K	[η]	K
In 1M CH_3COONa		
298.15	2.4941	-2.0680
303.15	1.5252	-2.8067
308.15	0.8470	-4.0989
313.15	1.1466	-3.5638
318.15	2.2007	-2.2623
323.15	1.9236	-2.5667
In 2M CH_3COONa		
298.15	1.8587	-2.3743
303.15	1.9709	-2.4158
308.15	3.0632	-1.6606
313.15	1.4267	-2.9769
318.15	1.3264	-2.6678
323.15	1.7372	-2.6301
In 1M CH_3COOK		
298.15	22.0780	-0.2584
303.15	16.2601	-0.3457
308.15	15.6918	-0.3593
313.15	14.2326	-0.3955
318.15	13.1939	-0.4224
323.15	13.5056	-0.4144

Table 2.4(a): B-Coefficient (dm^3mol^{-1}) of Aspartic acid in different salt solutions at different Temperatures.

Temp. K	1 M CH_3COONa	2M CH_3COONa	1M CH_3COOK
298.15	0.2862	0.3588	0.6720
303.15	0.2486	0.3602	0.5885
308.15	0.2185	0.3613	0.6059
313.15	0.2594	0.3370	0.5892
318.15	0.3035	0.4808	0.5165
323.15	0.4072	0.3621	0.5963

Table 2.4(b): B-Coefficient (dm^3mol^{-1}) of Glutamic acid in different salt solutions at different Temperatures.

Temp. K	1 M CH_3COONa	2M CH_3COONa	1M CH_3COOK
298.15	0.3111	0.4485	0.5121
303.15	0.3681	0.3598	0.6002
308.15	0.3873	0.3630	0.5458
313.15	0.3407	0.3527	0.5815
318.15	0.2979	0.4315	0.6377
323.15	0.2843	0.3308	0.6212

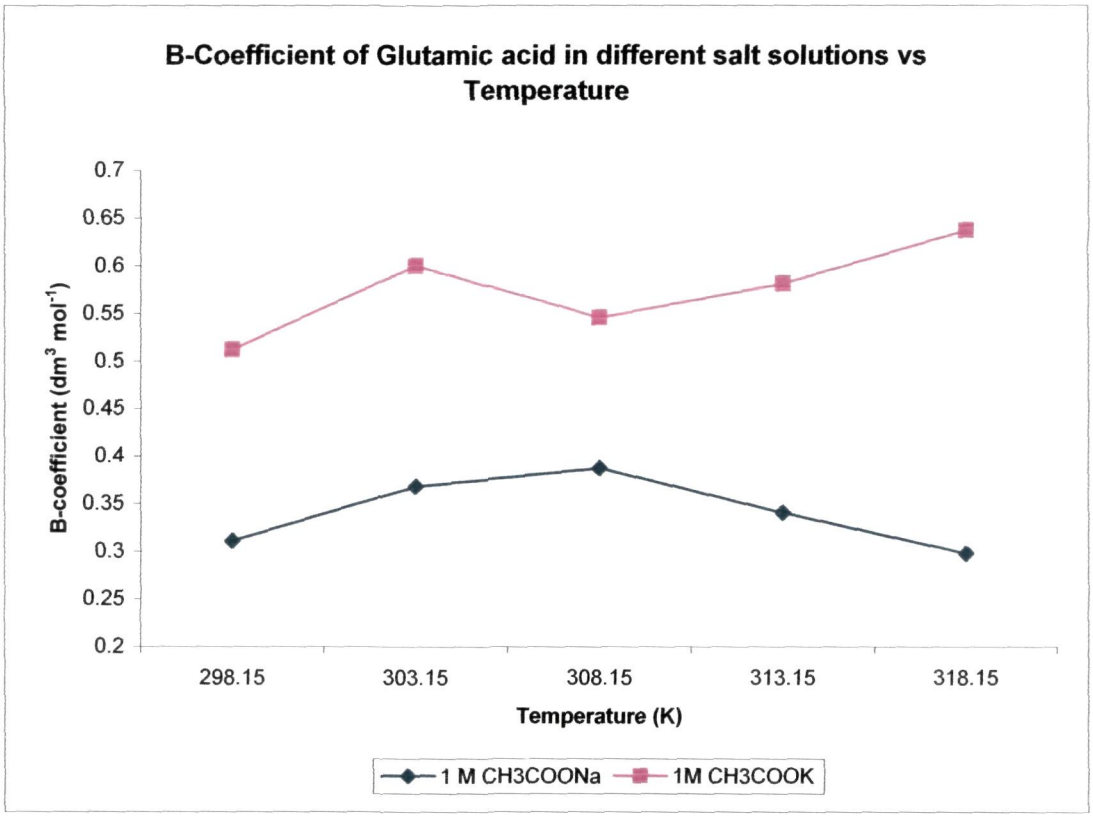
B-Coefficient of Glutamic acid in different salt solutions vs Temperature
B-coefficient (dm3 mol-1)
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
298.15
303.15
308.15
313.15
318.15
Temperature (K)
1 M CH3COONa
1M CH3COOK

Fig. 2.3

It is noted that at any given temperature in any of the cosolute, B-coefficient of glutamic acid is greater than that of aspartic acid. The significance of this order is that B-values increase with increasing alkyl chain length of the α-amino acids. It is well established that B-coefficient is a measure of solute-solvent interaction and is directly dependent on the size, shape and charge of the solute molecules [26,27,12]. Since the charged groups of the amino acids studied here are the same, the observed order of viscosity B-coefficients may just be explained in terms of the shape and size of the alkyl groups of the amino acids as glutamic acid has one more CH_2 group than aspartic acid

When CH_3COOK is cosolute, the values of B-coefficient are higher in comparison to its values when CH_3COONa is cosolute [Tables 2.4(a,b)], probably due to large size of K^+ ions than Na^+ ions.

At higher temperatures (above 308 K), the dB/dT values of CH_3COOK are positive while that of CH_3COONa are negative, probably bigger size of K^+ ion is responsible for creating disorder in the solvent structures [Fig. 2.3(a)].

Plotting $RT\ln(\eta V_m / hN)$ quantity vs T for each binary mixture [Figs. 2.4(a-f)], we have found that the plots show quite linear trend. On this basis one may suggest that the mechanism of viscous flow for these mixtures is a thermally activated single process.

The values of thermodynamic parameters (e.g., ΔG^*, ΔS^* and ΔH^*) are recorded in Tables 2.6(a,b). For all systems, the values for ΔG^* and ΔH^* are positive while ΔS^* values are negative.

Table 2.5(a): $RT\ln(\eta V_m/hN)$ ($kJmol^{-1}$) of Aspartic acid in 1M CM_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1471	0.1972
0.29815	65.0583	65.2384	65.4365	65.6304	65.8182
0.30315	65.8306	66.0035	66.1933	66.3978	66.5821
0.30815	66.6545	66.8206	67.0147	67.2146	67.4048
0.31315	67.4921	67.6779	67.8875	68.0844	68.2761
0.31815	68.2826	68.4768	68.7096	68.9021	69.0995
0.32315	69.1163	69.2945	69.5242	69.7748	69.9825

Table 2.5(b): $RT\ln(\eta V_m/hN)$ ($kJmol^{-1}$) of Glutamic acid in 1M CM_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1472	0.1972
0.29815	65.1396	65.3218	65.5443	65.7462	65.9661
0.30315	65.8561	66.0720	66.3023	66.5337	66.7170
0.30815	66.6787	66.8680	67.0868	67.3438	67.5615
0.31315	67.5187	67.7310	67.9554	68.1802	68.4062
0.31815	68.3471	68.5566	68.7964	69.0280	69.2145
0.32315	69.1703	69.3863	69.6112	69.8394	70.0555

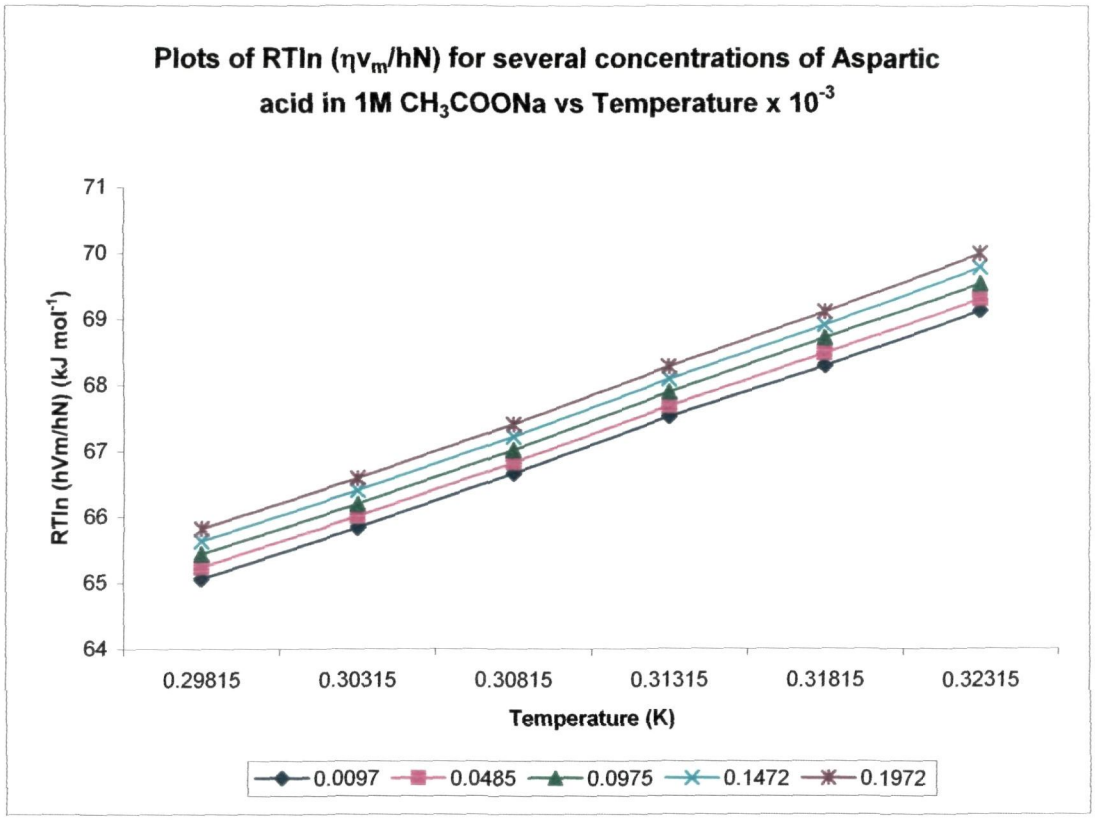

Fig. 2.4(a)

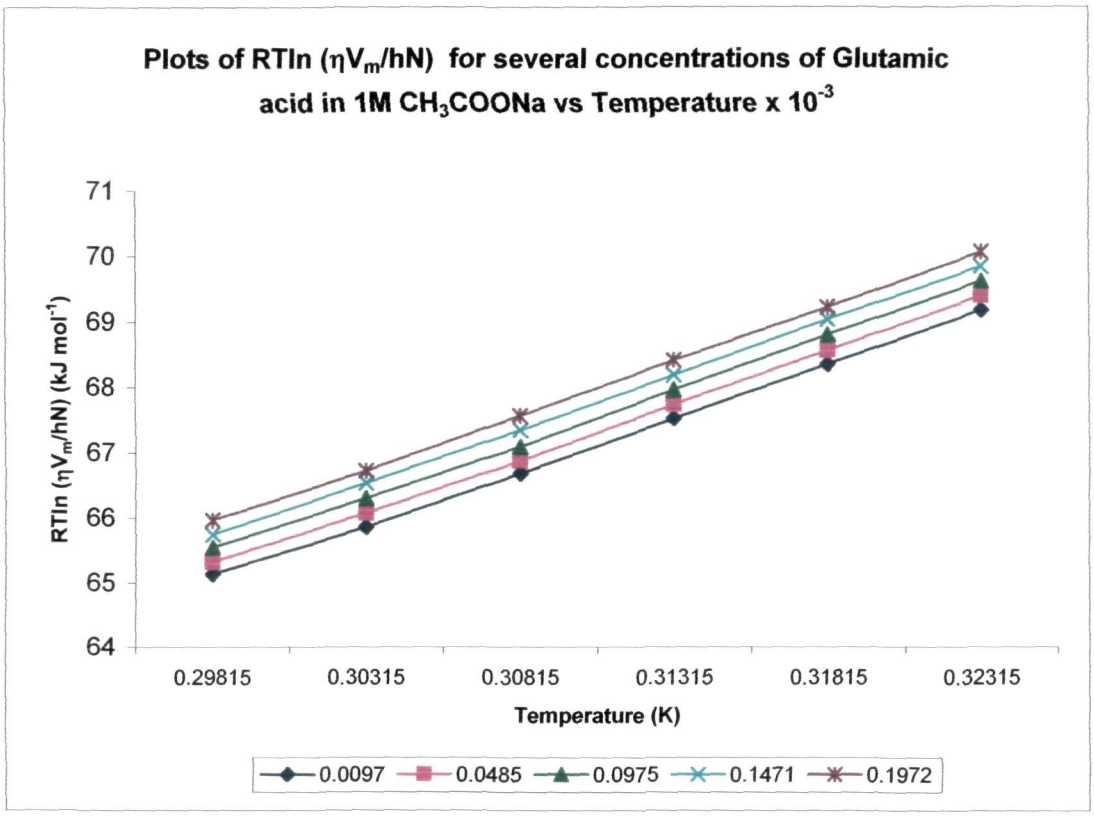

Fig. 2.4(b)

Table 2.5(c): $RTln(\eta v_m/hN)$ ($kJmol^{-1}$) of Aspartic acid in 2M CM_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0094	0.0470	0.0944	0.1425	0.1910
0.29815	67.4375	67.6186	67.7269	67.8321	67.9394
0.30315	68.2329	68.4177	68.5276	68.6355	68.7433
0.30815	69.0317	69.2641	69.3691	69.4693	69.5610
0.31315	69.9361	70.1160	70.2241	70.3387	70.4504
0.31815	70.7363	70.8555	70.9900	71.1605	71.3006
0.32315	71.5819	71.7705	71.8979	72.0082	72.1243

Table 2.5(d): $RTln(\eta v_m/hN)$ ($kJmol^{-1}$) of Glutamic acid in 2M CM_3COONa solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0094	0.0467	0.0945	0.1426	0.1912
0.29815	67.4936	67.6138	67.7660	67.9049	68.0426
0.30315	68.2916	68.4057	68.5439	68.6860	68.8084
0.30815	69.1265	69.2525	69.4090	69.5207	69.6599
0.31315	69.9721	70.0982	70.2638	70.3754	70.5104
0.31815	70.7585	70.9380	71.0861	71.2119	71.3565
0.32315	71.6324	71.7760	71.9182	72.0523	72.1789

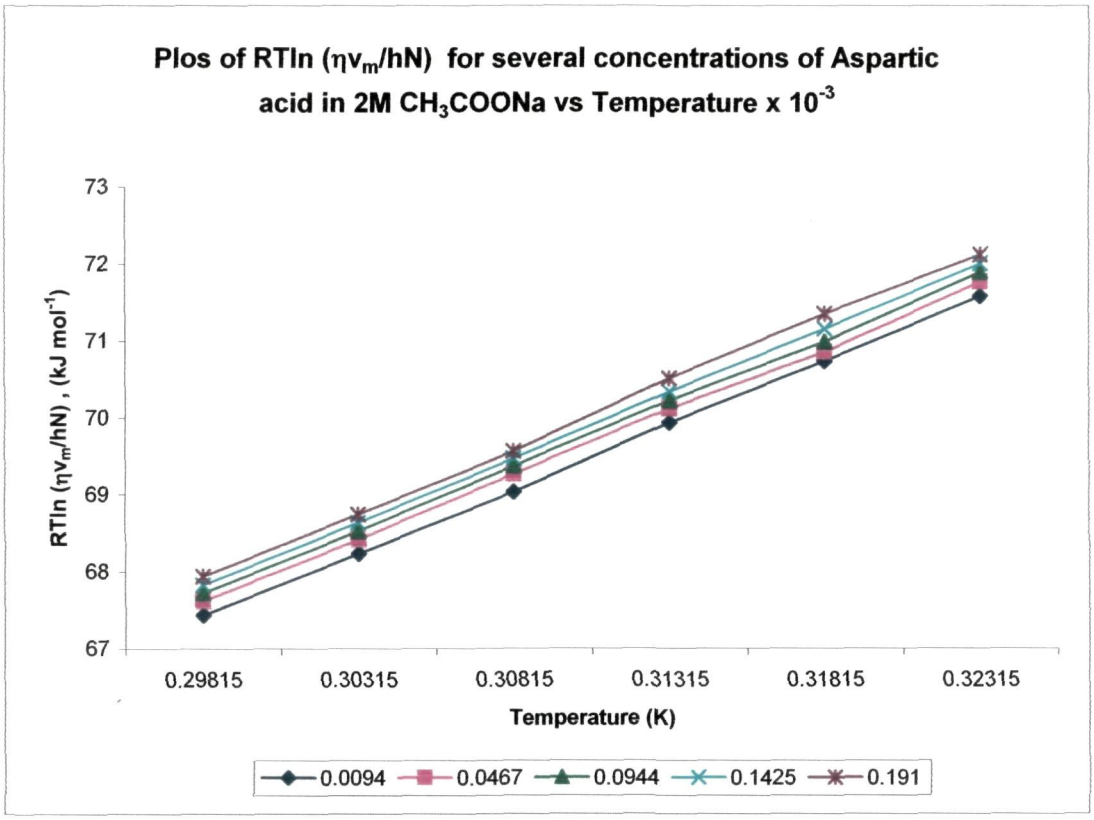

Fig. 2.4(c)

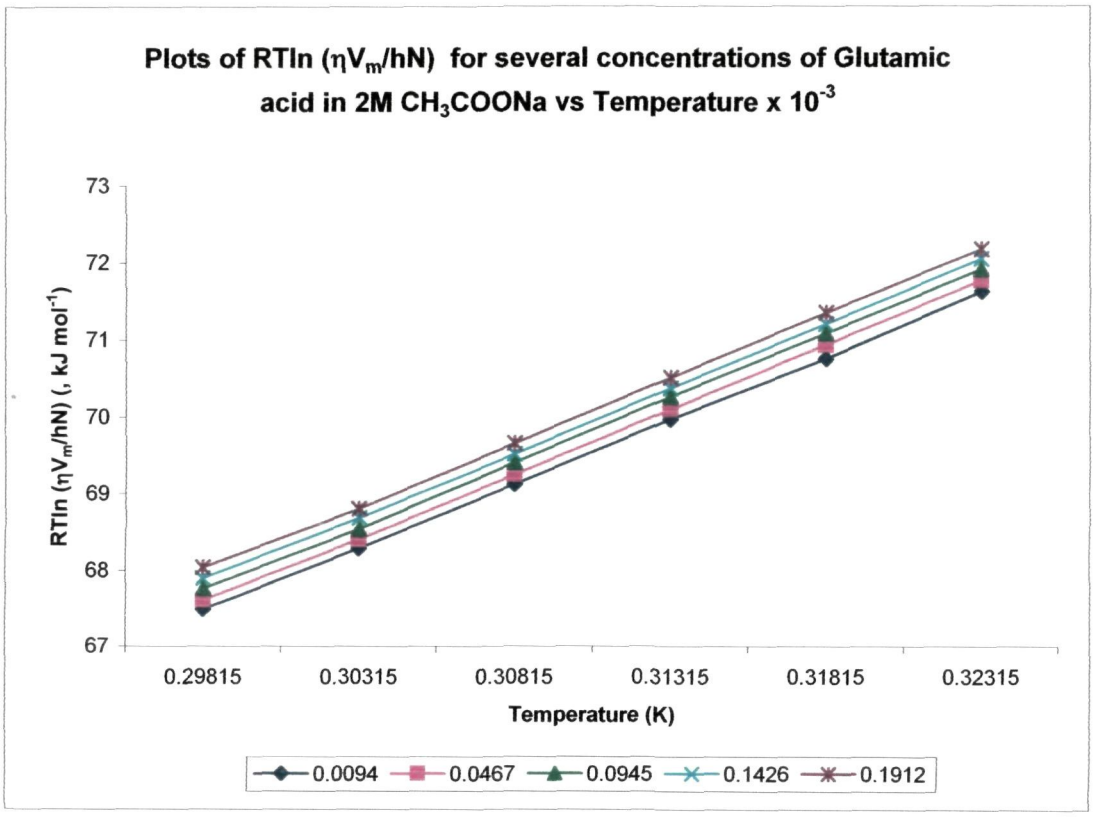

Fig. 2.4(d)

Table 2.5(e): $RTln(\eta v_m/hN)$ ($kJmol^{-1}$) of Aspartic acid in 1M CM_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0096	0.0481	0.0969	0.1461	0.1959
0.29815	65.2144	65.4082	65.5983	65.8033	65.9818
0.30315	65.9928	66.1630	66.3461	66.5670	66.7498
0.30815	66.7951	66.9777	67.1631	67.3921	67.5748
0.31315	67.6906	67.8316	68.0377	68.2968	68.4538
0.31815	68.4884	68.6770	68.8852	69.0954	69.2615
0.32315	69.3450	69.4986	69.7026	69.9332	70.1609

Table 2.5(f): $RTln(\eta v_m/hN)$ ($kJmol^{-1}$) of Glutamic acid in 1M CM_3COOK solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0096	0.0482	0.0965	0.1461	0.1959
0.29815	65.3209	65.4951	65.6982	65.8786	66.0867
0.30315	66.0200	66.2338	66.4024	66.6329	66.8490
0.30815	66.8435	67.0167	67.2539	67.4535	67.6477
0.31315	67.7092	67.8733	68.0922	68.3232	68.5431
0.31815	68.5256	68.7000	68.9696	69.1517	69.4069
0.32315	69.3671	69.5344	69.7932	70.0031	70.2478

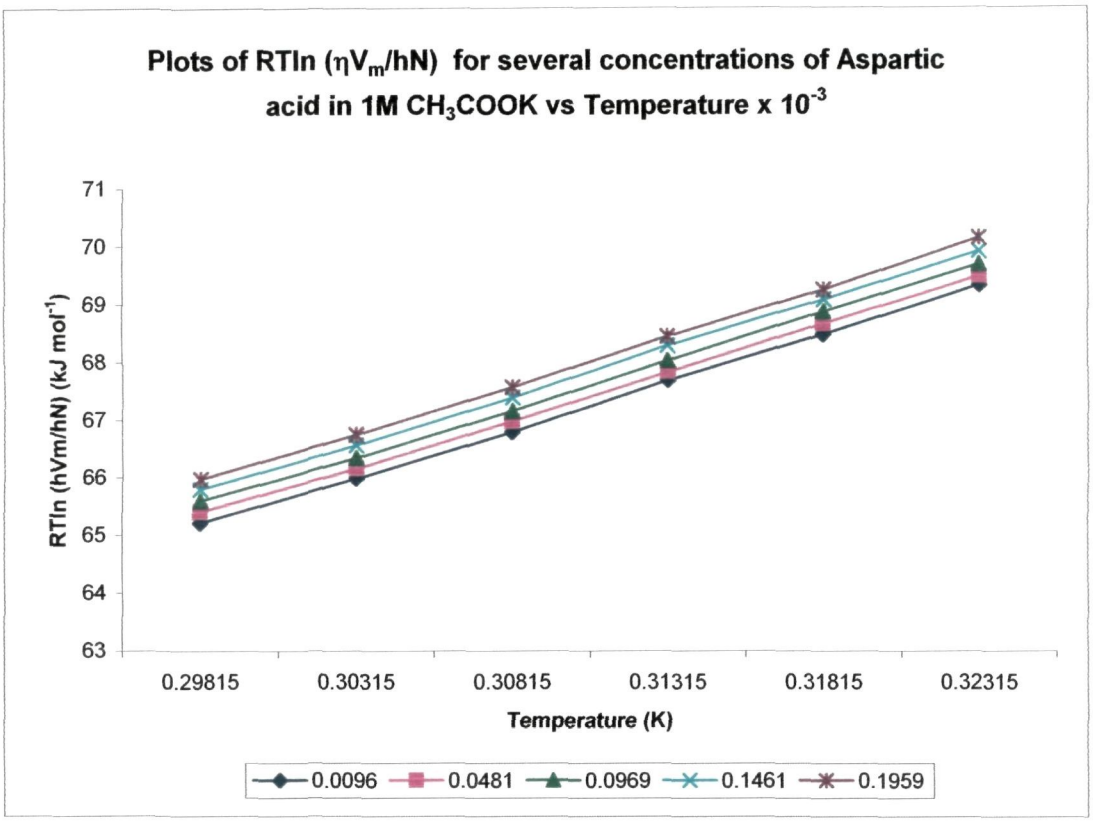

Fig. 2.4(e)

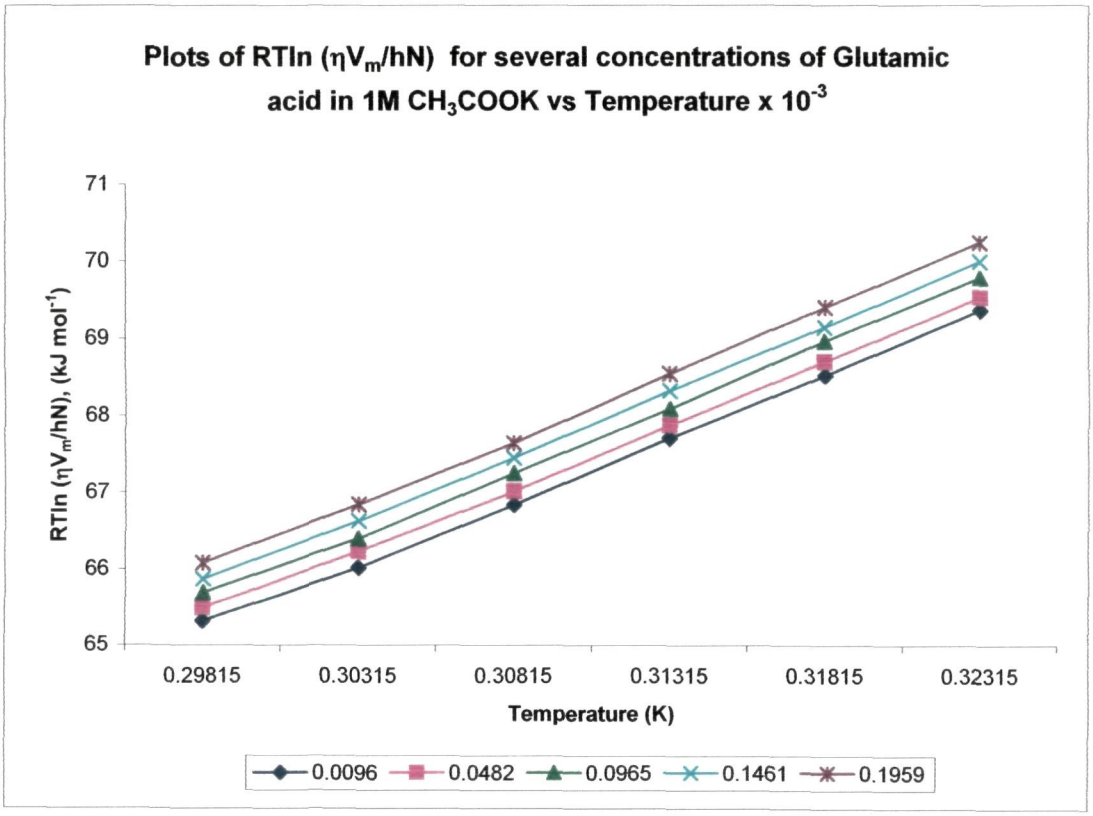

Fig. 2.4(f)

Table 2.6(a): Entropy (ΔS^*, kJ mol^{-1}) and Enthalpy (ΔH^*, kJ mol^{-1}) of Aspartic acid as a Function of Concentration in different salt solutions.

Molality (mol kg^{-1})	ΔS^*, kJ mol^{-1}	ΔH^*, kJ mol^{-1}
In 1M CH_3COONa		
0.0097	-125.5880	16.5099
0.0485	-141.8949	16.5580
0.0975	-148.8349	16.2300
0.1471	-153.0920	16.0024
0.1972	-155.8034	16.9465
In 2M CH_3COONa		
0.0094	-166.4949	17.7711
0.0470	-165.2846	18.3281
0.0944	-166.2697	18.1376
0.1425	-167.5709	17.8515
0.1910	-168.4903	17.6783
In 1M CH_3COOK		
0.0096	-165.9160	15.7125
0.0481	-164.8451	16.2169
0.0969	-165.7909	16.1192
0.1461	-166.5109	16.1214
0.1959	-167.3120	16.0560

Table 2.6(b): Entropy (ΔS^*, kJ mol^{-1}) and Enthalpy (ΔH^*, kJ mol^{-1}) of Glutamic acid as a Function of Concentration in different salt solutions.

Molality (mol kg^{-1})	ΔS^*, kJ mol^{-1}	ΔH^*, kJ mol^{-1}
In 1M CH_3COONa		
0.0097	-162.6657	16.5863
0.0485	-163.6531	16.4838
0.0975	-163.9166	16.6287
0.1471	-164.4874	16.6805
0.1972	-164.4811	16.8907
In 2M CH_3COONa		
0.0094	-165.3703	18.1735
0.0467	-164.3063	18.6556
0.0945	-167.0994	17.9217
0.1426	-166.6823	18.1787
0.1912	-166.7217	18.3007
In 1M CH_3COOK		
0.0096	-163.5057	16.5047
0.0482	-162.5811	16.9697
0.0965	-165.7994	16.1960
0.1461	-165.9920	16.3418
0.1959	-167.8549	15.9861

Table 2.7(a): Free Energy of Activation for viscous flow ΔG* (kJ mol–1) of several Concentrations of Aspartic acid in 1M sodium acetate at different Temperatures.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1471	0.1972
298.15	65.0378	65.2121	65.3995	65.5885	65.7716
303.15	65.8516	66.0281	66.2241	66.4201	66.6072
308.15	66.6654	66.8440	67.0487	67.2516	67.4428
313.15	67.4793	67.6599	67.8733	68.0832	68.2783
318.15	68.2931	68.4759	68.6978	68.9148	69.1139
323.15	69.1069	69.2918	69.5224	69.7463	69.9495

Table 2.7(b): Free Energy of Activation for viscous flow ΔG* (kJ mol–1) of several Concentrations of Glutamic acid in 1M CH_3COONa solution at different Temperatures.

Molality mol kg^{-1} / Temp. K	0.0097	0.0485	0.0975	0.1472	0.1972
298.15	65.0851	65.2770	65.5004	65.7224	65.9307
303.15	65.8984	66.0952	66.3200	66.5449	66.7531
308.15	66.7117	66.9135	67.1396	67.3673	67.5756
313.15	67.5251	67.7318	67.9592	68.1897	68.3980
318.15	68.3384	68.5500	68.7788	69.0122	69.2204
323.15	69.1517	69.3683	69.5984	69.8346	70.0428

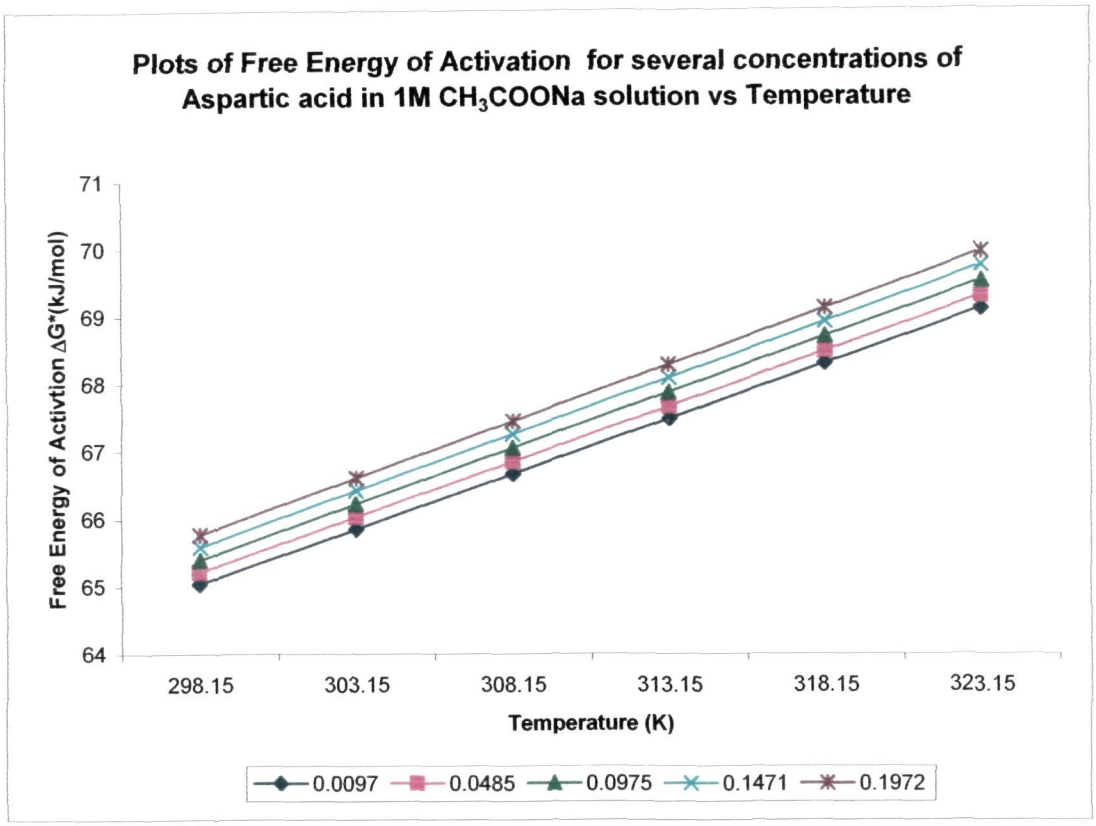

Fig. 2.5(a)

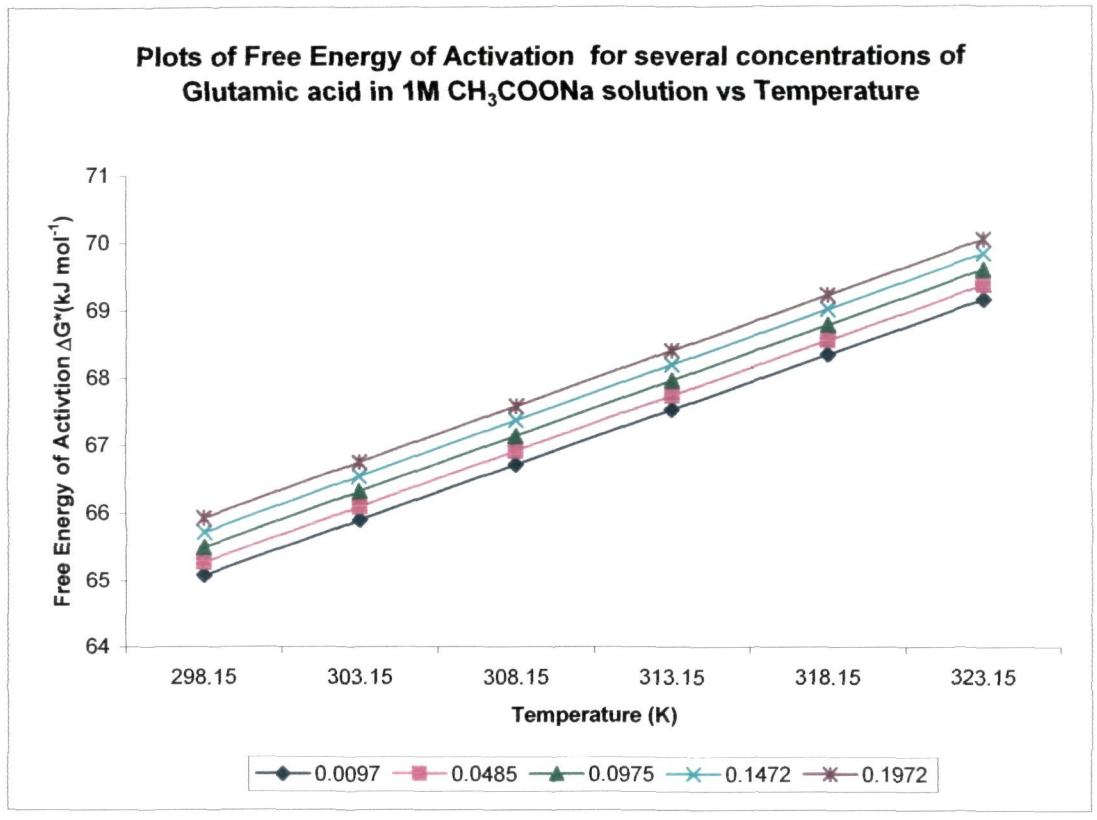

Fig. 2.5(b)

Table 2.7(c): **Free Energy of Activation for viscous flow ΔG* (kJ mol–1) of several Concentrations of Aspartic acid in 2M CH_3COONa solution at different Temperatures.**

Molality mol kg^{-1} / Temp. K	0.0094	0.0470	0.0944	0.1425	0.1910
298.15	67.4116	67.6077	67.7109	67.8128	67.9137
303.15	68.2440	68.4341	68.5423	68.6506	68.7561
308.15	69.0765	69.2606	69.3736	69.4885	69.5986
313.15	69.9080	70.0870	70.2050	70.3263	70.4410
318.15	70.7415	70.9134	71.0363	71.1642	71.2835
323.15	71.5739	71.7389	71.8677	72.0020	72.1259

Table 2.7(d): **Free Energy of Activation for viscous flow ΔG* (kJ mol–1) of several Concentrations of Glutamic acid in 2M CH_3COONa solution at different Temperatures.**

Molality mol kg^{-1} / Temp. K	0.0094	0.0467	0.0945	0.1426	0.1912
298.15	67.4787	67.6435	67.7424	67.8750	68.0088
303.15	68.3055	68.4651	68.5779	68.7084	68.8424
308.15	69.1324	69.2866	69.4134	69.5418	69.6760
313.15	69.9592	70.1081	70.2489	70.3753	70.5096
318.15	70.7861	70.9296	71.0844	71.2087	71.3432
323.15	71.6129	71.7512	71.9199	72.0721	72.1768

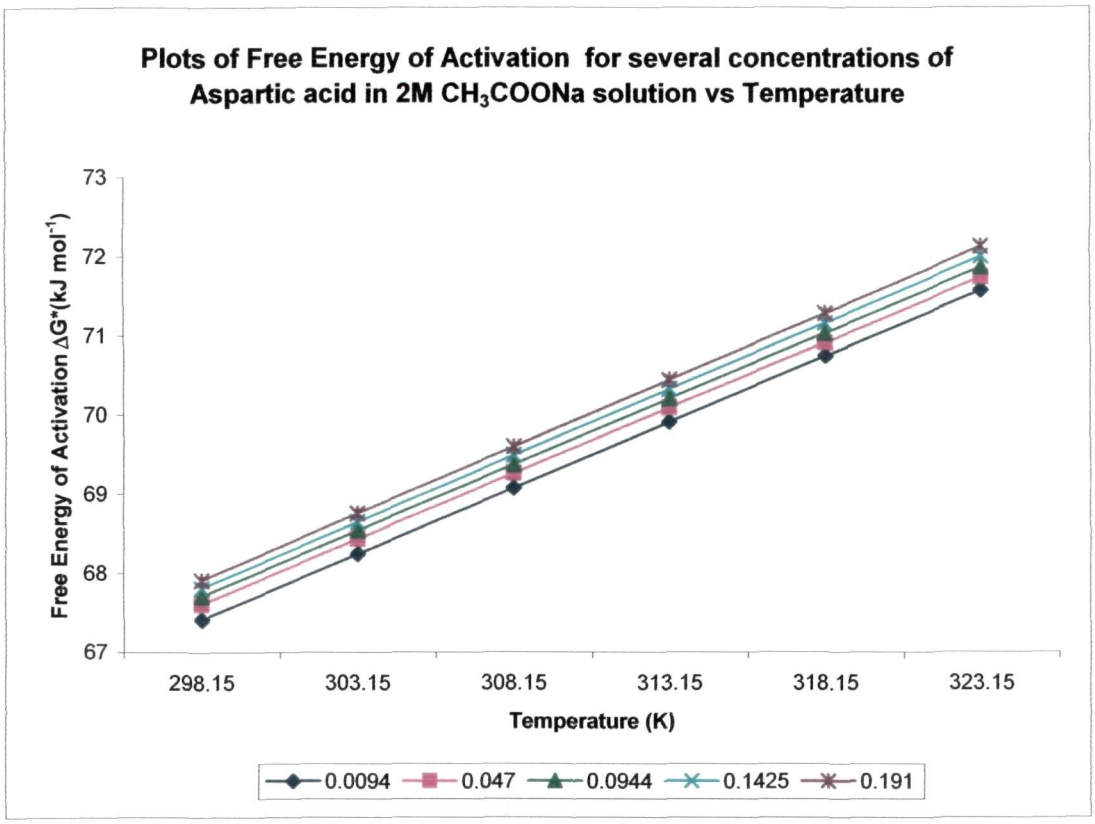

Fig. 2.5(c)

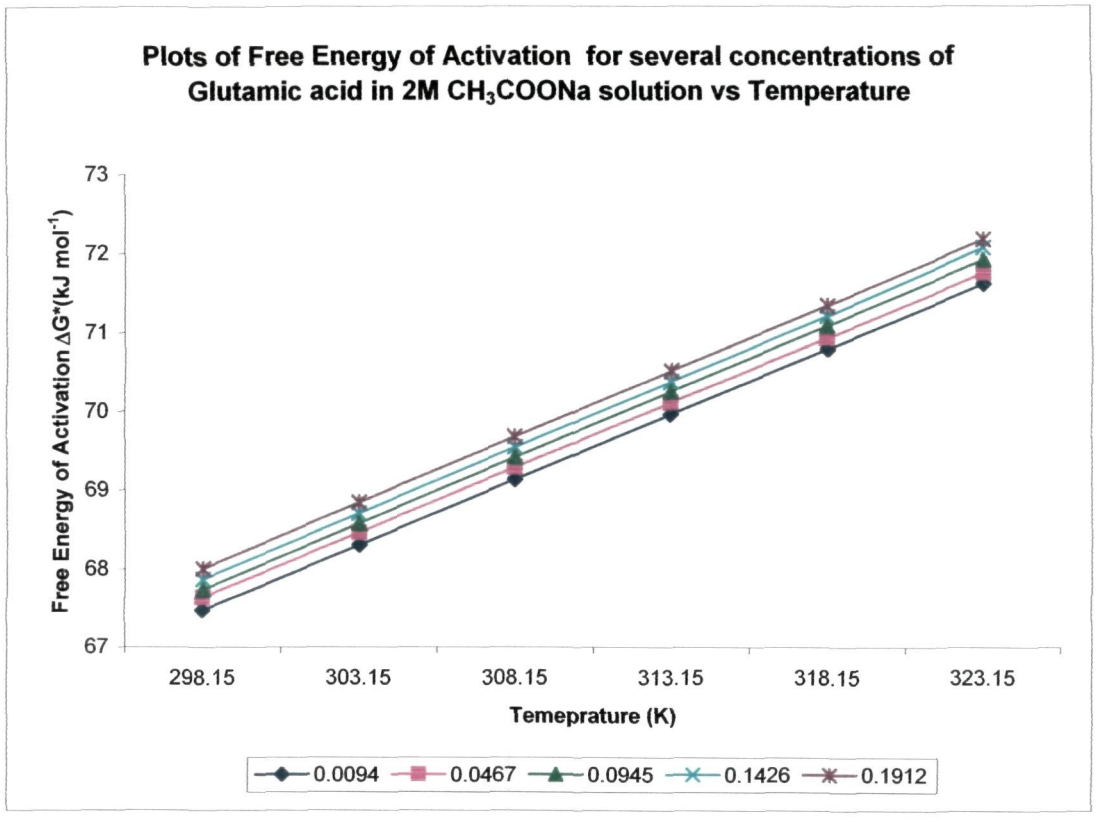

Fig. 2.5(d)

Table 2.7(e): Free Energy of Activation for viscous flow ΔG* (kJ mol–1) of several Concentrations of Aspartic acid in 1M CH_3COOK solution at different Temperatures.

Molality mol kg^{-1} / Temp. K	0.0096	0.0481	0.0969	0.1461	0.1959
298.15	65.1805	65.3655	65.5498	65.7666	65.9401
303.15	66.0100	66.1897	66.3787	66.5992	66.7766
308.15	66.8396	67.0139	67.2077	67.4317	67.6132
313.15	67.6692	67.8381	68.0366	68.2643	68.4498
318.15	68.4988	68.6624	68.8656	69.0968	69.2863
323.15	69.3284	69.4866	69.6945	69.9294	70.1229

Table 2.7(f): Free Energy of Activation for viscous flow ΔG* (kJ mol–1) of several Concentrations of Glutamic acid in 1M CH_3COOK solution at different Temperatures.

Molality mol kg^{-1} / Temp. K	0.0096	0.0482	0.0965	0.1461	0.1959
298.15	65.2539	65.4433	65.6291	65.8323	66.0320
303.15	66.0715	66.2562	66.4581	66.6623	66.8713
308.15	66.8890	67.0691	67.2871	67.4922	67.7106
313.15	67.7065	67.8820	68.1161	68.3222	68.5495
318.15	68.5240	68.6949	68.9451	69.1522	69.3891
323.15	69.3416	69.5078	69.7741	69.9821	70.2284

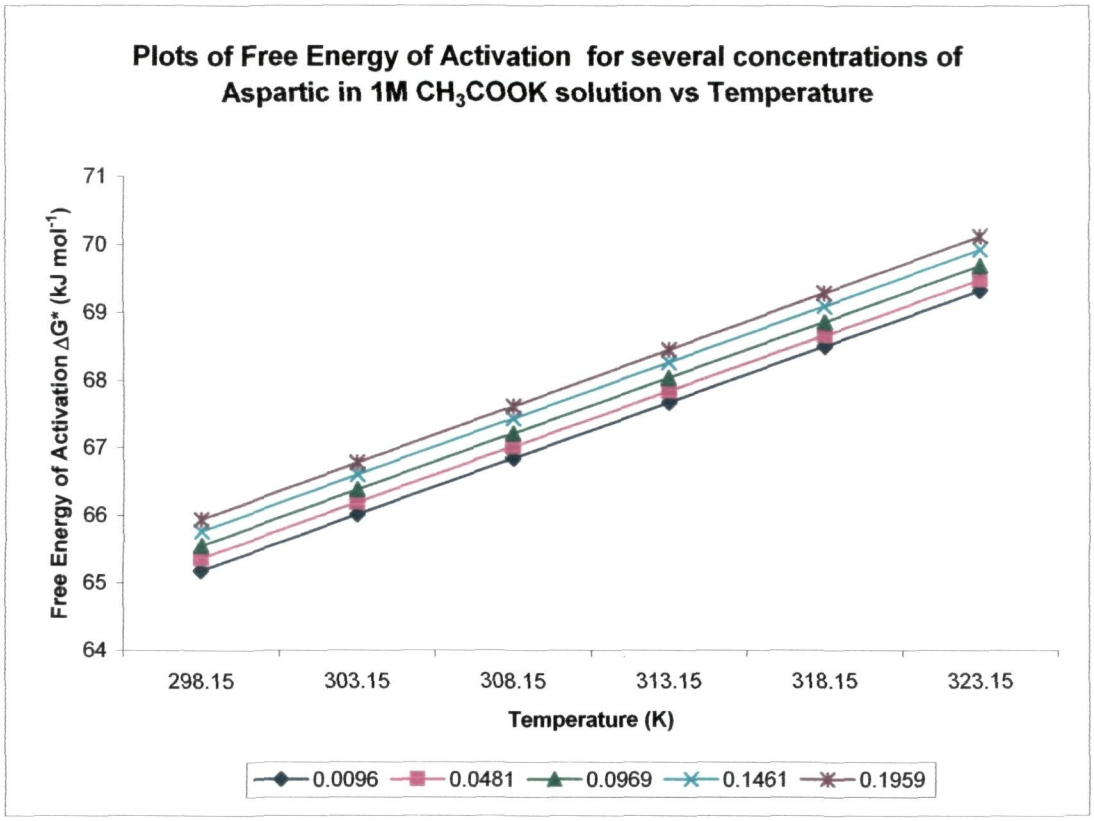

Fig. 2.5(e)

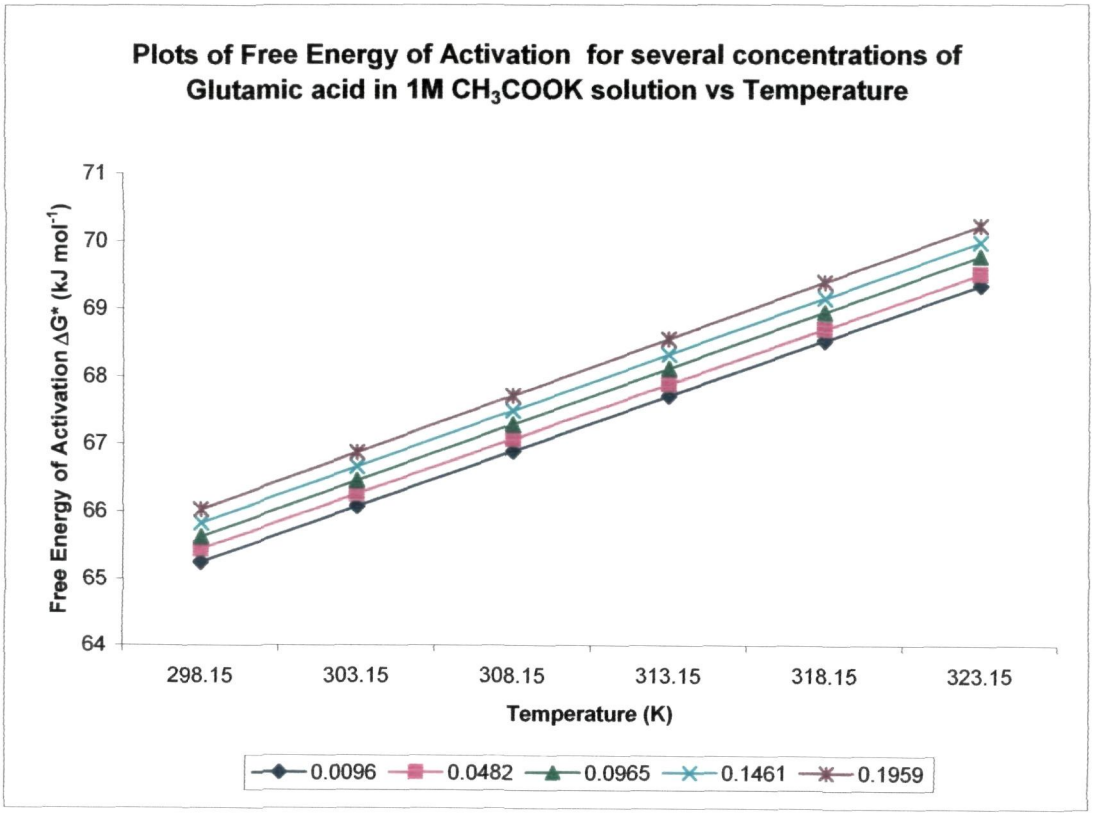

Fig. 2.5(f)

An activation energy of viscous flow ΔG^* can be interpreted within the frame of applications of the absolute rate theory to the process of flow [13].

In this theory, the activation energy of a reaction is defined as the additional energy, which reactant molecules must acquire in order to form the intermediate activated complex for the reaction. The reacting molecules possessing sufficient energy approach each other and there is redistribution of energy and bonds so that the activated complex acquires energy to form the intermediate bond. The positive values of ΔG^* show that the formation of the transition state is less favoured in the presence of these systems, meaning thereby that the formation of the transition state is accompanied by the breaking and distortion of intermolecular bonds.

A satisfactory elucidation of these facts probably arises from the more realistic hypothesis of the flow mechanism of Eyring [28], which explains the flow by movement of dislocations or discontinuities in the fluid layers. In a dynamic steady state, and in an over simplified picture, the movement of a dislocation by one layer position requires the cooperation of at least two moving elementary units: one is moving out the standard position and requires energy, and the other is moving into this cavity and gives up energy. Therefore, the enthalpy of activation of viscous flow could be taken as a measure of the cooperation degree between the species taking part in the flow process. Actually, in the liquid state the opportunity of the formation of many discontinuities is warranted by statistical fluctuations of local density. In the low

temperature range as well as for highly structured components, one may expect a considerable degree of order, so that transport phenomenon takes place cooperatively, as a consequence a great heat of activation associated with a relatively high value of flow entropy is observed. When the breaking in the ordered and polymerized fluid structure becomes very quick, by increasing the temperature or by adding a component that breaks a homopolymer hydrogen bond network, the movement of the individual units becomes more disordered and the cooperation degree is reduced, facilitating the viscous flow via the activated state of molecular species. As a consequence the overall molecular order in the system should be reduced and positive ΔS^* values should be expected.

The evidence obtained in this work appears quite intriguing because at higher temperatures as well as in the high solute region, the availability of randomly scattered monomers should be sufficient to provide the activated molecular species which, then leads to comparatively increased order as a result of viscous flow, giving the more negative ΔS^* values [Tables 2.6(a,b)].

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Chapter-3

Effect of Glucose, Maltose and Urea on the stabilization of Lysozyme solution in terms of volumetric and Adiabatic compressibility behaviour

Globular proteins, nature's most functionally diverse macromolecules, are only marginally stable. Even under physiological conditions, a delicate balance of forces combine to give proteins maximum stability of only a few kilocalories per mole [1], a value near the dissociation energy of a few hydrogen bonds [2]. Solutes can have large effects on proteins [3]. For instance, urea [4,5], urea derivatives [6], and guanidium chloride, denature proteins while sugars [7,8] and glycine derivatives [7,9] can double a protein's stability. It is important to understand the mechanism by which solutes exert these large effects.

Lysozyme is an enzyme with antibacterial action that is found in body fluids. An enzyme is a protein or conjugated protein produced by a living organism and functions as a biological catalyst [10]. Lysozyme breaks down cell walls and kills bacteria. Lee-Huang et al. reported that lysozyme is also an effective agent for killing HIV *in vitro* [11]. It is a suitable model system for investigating crystallization of biomolecules since it fulfills some requirements:

(i) The protein is stable and its monomeric state is well defined and

(ii) Aggregation and concomitant crystallization can be easily induced by simple electrolytes, i.e., NaCl, upon screening the net positive surface charges [12].

It is a relatively small protein (14306D_a) that is highly amenable to direct analysis. It is a single polypeptide chain of 129 residues. This highly stable protein is cross-linked by four disulfide bridges. The interior of lysozyme, like that of myoglobin and

hemoglobin is almost entirely non-polar. Hydrophobic interaction plays an important role in the folding of lysozyme [13].

In living organisms, interactions of carbohydrates with proteins play a key role in a wide range of biochemical processes. In particular, carbohydrates located at cell surface are receptors with regard to the bioactive structures of hormones, enzymes, viruses, antibodies, etc. [14]. Therefore, the studies of carbohydrate-protein interactions are very important for immunology, biosynthesis, pharmacology, and medicine. Analysis of literature data shows that general information about the interaction between carbohyderates and proteins could be obtained from X-ray crystallography [15-18], NMR spectral [19], computer calculations [20-22] and chromatography data [23,24]. There are also investigations devoted to the kinetics of these interactions [25, 26].

Aqueous urea and its derivatives are important mixed solvents. They have been the subject of numerous investigations, which have ranged widely in scope and purpose [27-28].

Urea and its derivatives are well characterized in water and act as a statistical structure breaker. From the results of interaction of guanidine hydrochloride, urea and its alkyl derivatives upon the structure of water, it was concluded that guanidine hydrochloride and urea molecules behave as a structure breaker for liquid water [29-33]. The structure of urea + water mixture is of great importance in understanding the protein denaturation.

The activity of egg-white lysozyme was measured in the presence of carbohydrate additives (glucose and maltose) and urea in the reaction medium by accurate quantitative measurements of such properties as density, ρ and compressibility β [34] as functions of concentration of sugars or urea (keeping the concentration of aqueous lysozyme solution constant) and temperature. The interaction of protein with water molecules in aqueous sugar and urea solutions and the temperature dependence of these interactions play very important role in understanding the thermodynamic processes in living cells.

Results and Discussion

The density measurements were performed with a calibrated pyknometer at different temperatures ranging from 20°C to 50°C with an accuracy of ±0.01°C. The density data of lysozyme + (sugar or urea) + water system are given in Tables 3.1(a-c) for several concentrations at different temperatures. The values of density of these systems increase and decrease with different compositions. The change in structure of solvent or solution as a result of H-bond formation or disruption leads to decrease or increase in intermolecular free length. Structure making or structure breaking character of the solute, i.e., H-bond forming or disrupting properties are thus correlated to change in density. Solutes can occupy the interstitial spaces (cavities) of solvents. Sugars increase the density of water and urea decreases the density.

From the density data, the apparent molal volumes, V_ϕ of sugar or urea in lysozyme solution are calculated from equation (1),

$$V_\phi = \frac{M}{\rho} - \frac{1000(\rho - \rho_0)}{ma\rho\ \rho_0} \qquad (1)$$

where V_Φ is the apparent molal volume of solute, ρ_0 and ρ are the densities of solvent and solution, respectively, m_a is the molality and M represents the molecular weight of the solute.

The positive values of V_ϕ in all the systems indicate greater solute-solvent interactions. The value of apparent molal volume increases with increase in concentration of glucose or maltose. Such behaviour indicates that the solute-solvent interaction increases with increase in the concentration of glucose or maltose. In maltose, two glucose units are joined by α-1,4 glycosidic linkage, so the V_ϕ values of maltose + lysozyme + water system are expected to be nearly twice in comparison to glucose + lysozyme + water system [Tables 3.2 (a,b)]. The values of V_ϕ decrease with increase in urea concentration [Table 3.2(c)]. Such behaviour indicates that the solute-solvent interaction decreases with increase in the concentration of urea in solution.

V_Φ is a linear function of the concentration term and is in good agreement with Masson's equation [35-37],

$$V_\phi = V_\phi^0 + S_v m \qquad (2)$$

in which V_ϕ^0 is the partial molal volume at infinite dilution and is a measure of solute-solvent interaction [38]. It is obtained from the linear plot of V_ϕ vs m_a using the least-squares method. S_v is the experimental slope, which is sometimes considered to be a volumetric pair-wise interaction coefficient [39, 40]. It provides information on solute-solute interactions. There is no completely

Table 3.1(a): Densities, ρ(gm cm^{-3}) of D(-)Glucose in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0610	0.0810	0.1010
293.15	1.00091	1.00225	1.00357	1.00495	1.00625	1.00754
298.15	0.99873	1.00007	1.00139	1.00277	1.00407	1.00536
303.15	0.99651	0.99785	0.99917	1.00055	1.00185	1.00314
308.15	0.99452	0.99586	0.99718	0.99856	0.99986	1.00115
313.15	0.99234	0.99368	0.99500	0.99638	0.99768	0.99897
318.15	0.99065	0.99199	0.99331	0.99469	0.99599	0.99728
323.15	0.98881	0.99015	0.99147	0.99285	0.99415	0.99544

Table 3.1(b): Densities, ρ(gm cm^{-3}) of Maltose in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0610	0.0820	0.1030
293.15	1.00091	1.00352	1.00610	1.00878	1.01143	1.01405
298.15	0.99873	1.00134	1.00392	1.00660	1.00925	1.01187
303.15	0.99651	0.99912	1.00170	1.00438	1.00703	1.00965
308.15	0.99452	0.99713	0.99971	1.00239	1.00504	1.00766
313.15	0.99234	0.99495	0.99753	1.00021	1.00286	1.00548
318.15	0.99065	0.99326	0.99584	0.99852	1.00117	1.00379
323.15	0.98881	0.99142	0.99400	0.99668	0.99933	1.00195

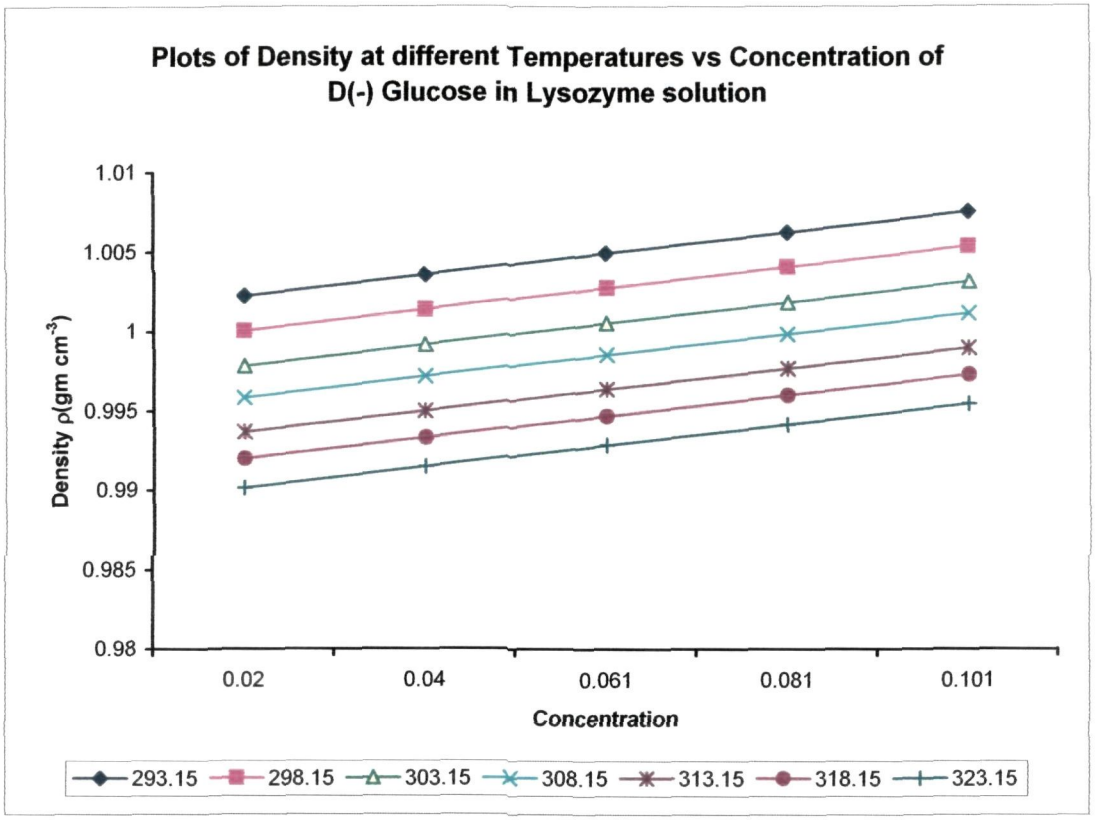

Fig. 3.1(a)

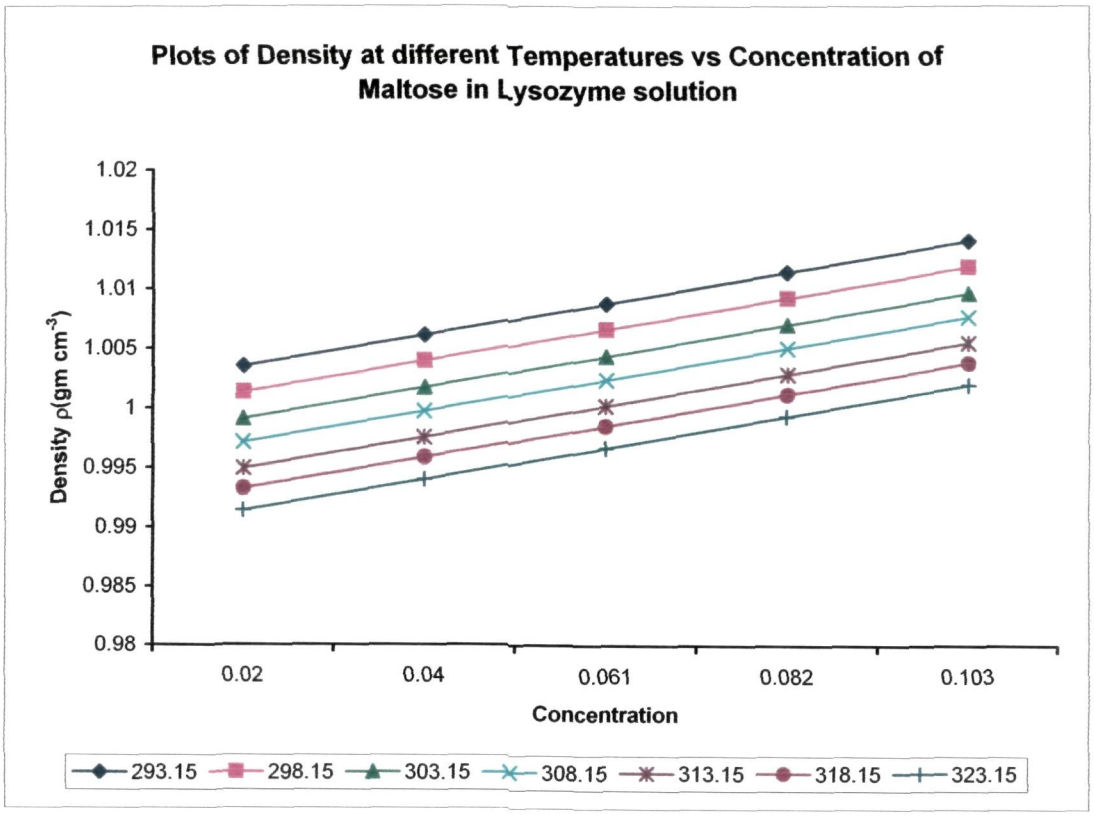

Fig. 3.1(b)

Table 3.1(c): Densities, ρ(gm cm^{-3}) of Urea in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0600	0.0800	0.1000
293.15	1.00091	1.00042	1.00000	1.00002	1.00004	1.00005
298.15	0.99873	0.99824	0.99782	0.99784	0.99786	0.99787
303.15	0.99651	0.99602	0.99560	0.99562	0.99564	0.99565
308.15	0.99452	0.99403	0.99361	0.99363	0.99365	0.99366
313.15	0.99234	0.99185	0.99143	0.99145	0.99147	0.99148
318.15	0.99065	0.99016	0.98974	0.98976	0.98978	0.98979
323.15	0.98881	0.98832	0.98790	0.98792	0.98794	0.98795

Table 3.2(a): Apparent Molal Volume, V_ϕ (cm^3 mol^{-1}) of D(-) Glucose in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0810	0.1010
293.15	112.97	113.32	113.43	113.58	113.72
298.15	113.07	113.42	113.53	113.69	113.82
303.15	113.17	113.52	113.64	113.79	113.93
308.15	113.26	113.61	113.73	113.89	114.02
313.15	113.36	113.72	113.83	113.99	114.13
318.15	113.44	113.79	113.91	114.07	114.21
323.15	113.52	113.88	114.00	114.16	114.29

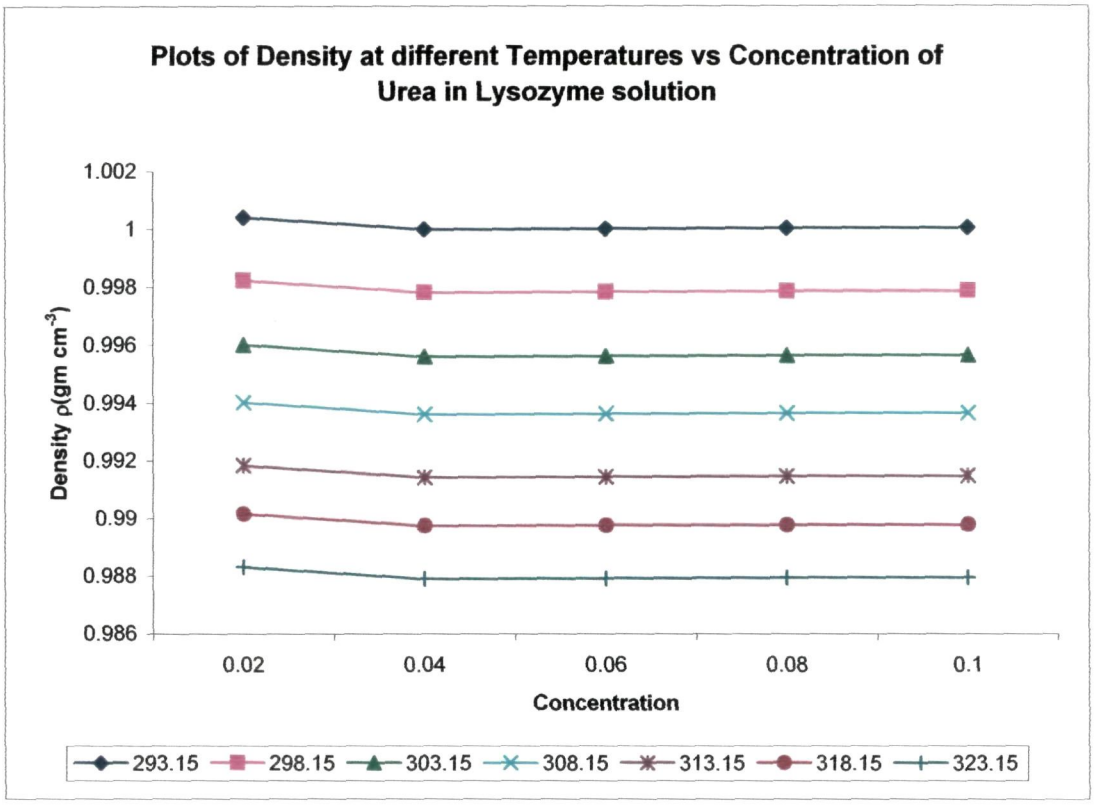

Fig. 3.1(c)

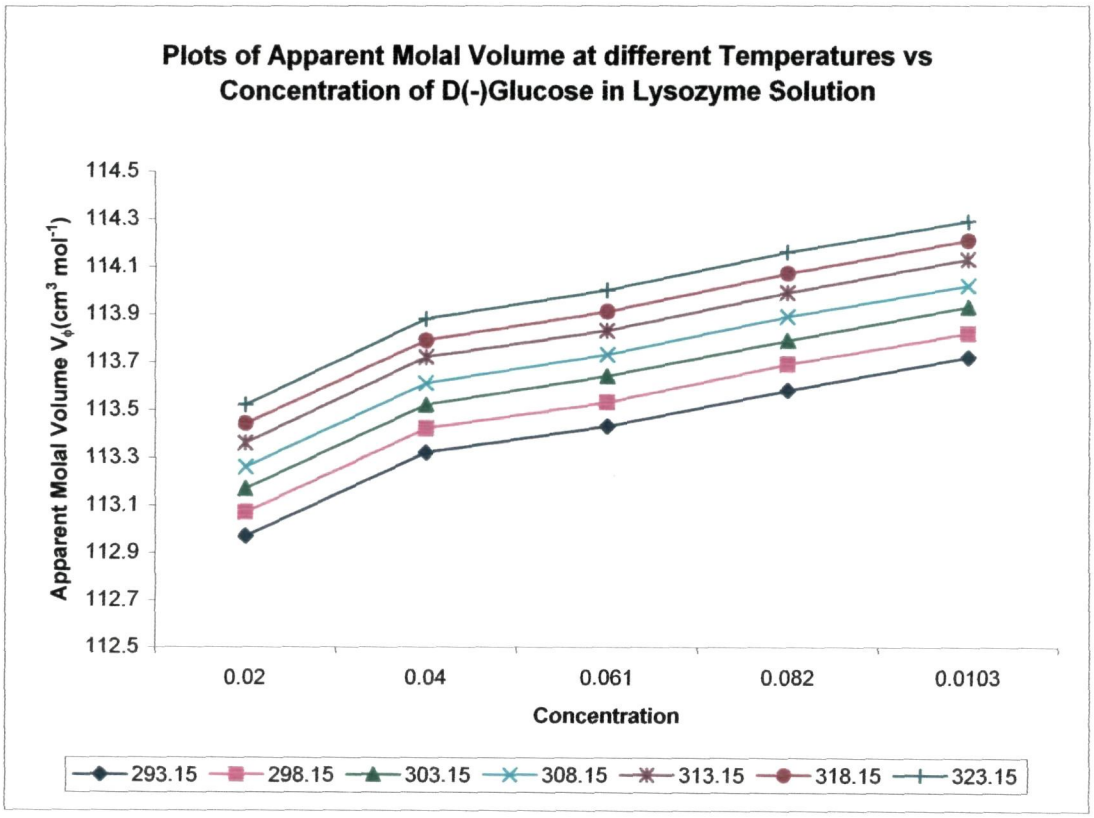

Fig. 3.2(a)

Table 3.2(b): Apparent Molal Volume, V_ϕ (cm^3 mol^{-1}) of Maltose in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0820	0.1030
293.15	229.13	229.29	229.41	229.52	229.64
298.15	229.35	229.51	229.62	229.74	229.86
303.15	229.57	229.73	229.85	229.96	230.08
308.15	229.76	229.92	230.04	230.16	230.28
313.15	229.97	230.14	230.26	230.38	230.50
318.15	230.14	230.30	230.43	230.55	230.67
323.15	230.32	230.48	230.61	230.73	230.85

Table 3.2(c): Apparent Molal Volume, V_ϕ (cm^3 mol^{-1}) of Urea in aqueous Lysozyme solution as Functions of Concentration and Temperatures.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0600	0.0800	0.1000
293.15	84.50	82.79	74.88	70.92	68.65
298.15	84.74	83.02	75.07	71.10	68.82
303.15	84.98	83.26	75.27	71.28	68.99
308.15	85.20	83.47	75.46	71.45	69.15
313.15	85.45	83.70	75.65	71.63	69.32
318.15	85.63	83.89	75.81	71.77	69.45
323.15	85.84	84.08	75.98	71.93	69.60

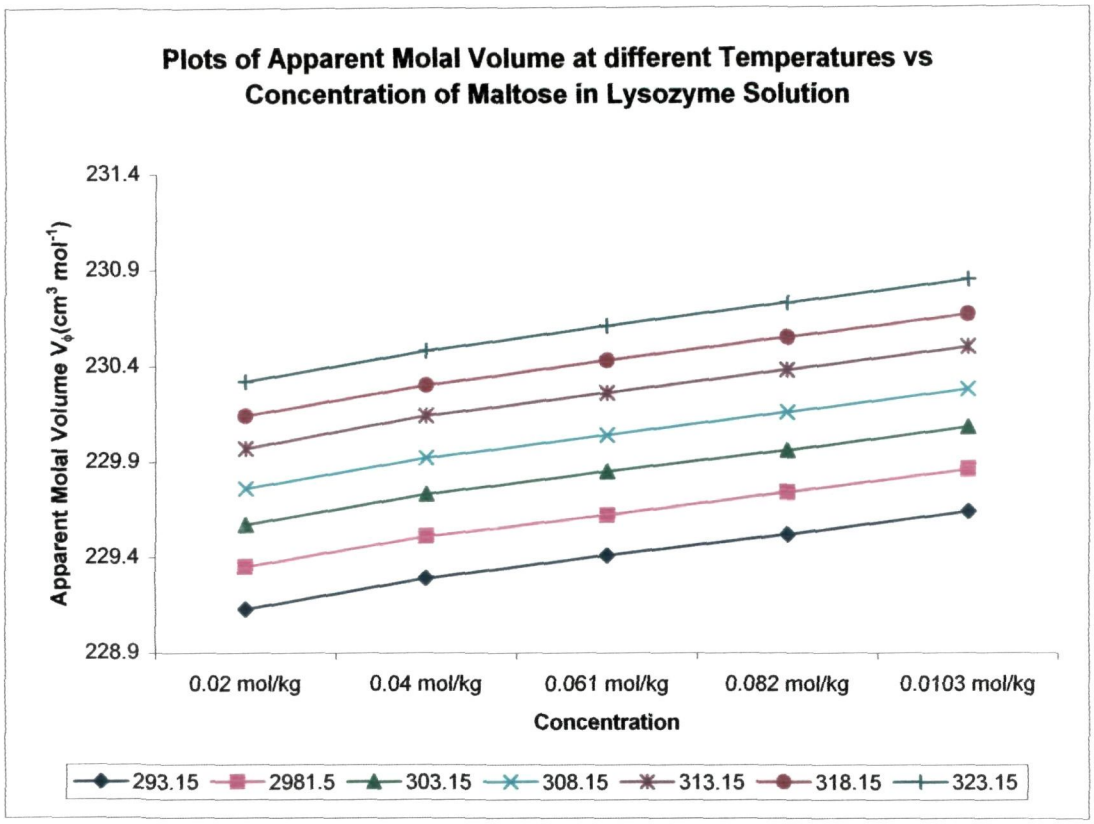

Fig. 3.2(b)

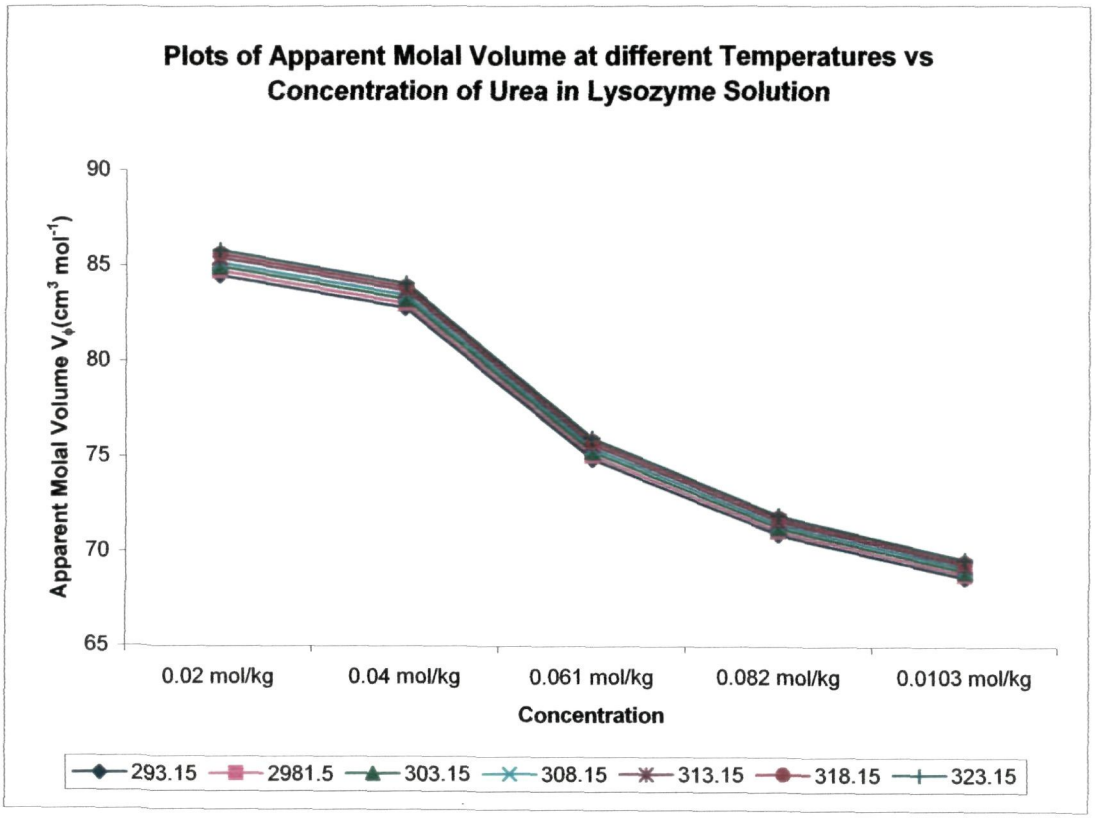

Fig. 3.2(c)

unambiguous numerical measure of "Structure making" or "Structure breaking" by a solute, but since a change in the extent to which water is structured is accompanied by a volume change [41], the partial molal volume of the solute provides a rough measure of the effect of that solute on the structure of water. The partial molal volume is the intrinsic volume of the solute molecule plus a contribution from whatever volume change results from the interaction of the solute with the surrounding solvent.

It is evident from Table 3.3(a) that the values of the slope, S_v for urea in urea + lysozyme + water system are negative at all temperatures suggesting weak solute-solute interaction in the system, while values of S_v are positive for sugars in sugar + lysozyme + water system suggesting strong solute-solute interaction.

Different models have been used to explain partial molal volume data. Franks et al [42] have shown that the partial molal volume of a non-electrolyte is a combination of the intrinsic volume of non-electrolyte and the volume due to its interaction with the solvent. The intrinsic volume has been considered to be made up of two types of contributions,

$$V_{\text{int}} = V_{vw} + V_{void}\,.$$

Shahidi et al [43] modified this equation to include the contribution of interaction of a non-electrolyte solute with the solvent,

$$V_{\phi}^{0} = V_{vw} + \vee_{void} - n\sigma_{s}$$

where σ_s is the shrinkage in volume produced by the interactions of hydrogen bonding groups present in the solute with water molecules and n is the potential number of hydrogen bonding sites in the molecule. For electrolytes and zwitterionic solutes, the shrinkage is caused by electrostriction and finally V_ϕ^0 can be evaluated using the relation,

$$V_\phi^0 = V_{vw} + V_{void} - V_{\text{shrinkage}}.$$

It has been assumed that V_{vw} and V_{void} have the same magnitude in water and in mixed aqueous solvents for the same class of compounds [44].

Transfer volumes of sugars $V_{\phi tr}^0$ from aqueous lysozyme to water solutions at infinite dilution at different temperatures are calculated from equation,

$$V_{\phi tr}^0 = V_{\phi\,(\text{in aq Lysozyme solution})}^0 - V_{\phi(in\,water)}^0.$$

The observed positive values of $V_{\phi tr}^0$ can be attributed to the decrease in volume of shrinkage and negative values of $V_{\phi tr}^0$ are due to the increase in volume of shrinkage in the presence of solutes. This may be attributed to various types of interactions occurring between glucose or maltose or urea with lysozyme molecules, which will have different contributions to $V_{\phi tr}^0$.

Hydrophilic-hydrophilic interactions between the -OH groups of monosaccharide and hydrophilic R group present on the exterior of lysozyme structure are responsible for the positive values of transfer values [Table 3.4] while the nonpolar-hydrophilic interactions between the nonpolar urea and

Table 3.3(a): Partial Molal Volume, V_ϕ^0 ($cm^3\ mol^{-1}$) of Sugars and Urea in aqueous Lysozyme solution at different Temperatures.

Temp. K	D-Glucose		Maltose		Urea	
	ϕ_v^0	S_v	ϕ_v^0	S_v	ϕ_v^0	S_v
293.15	112.88	8.67	229.03	6.00	89.42	-217.85
298.15	112.98	8.72	229.25	6.01	89.68	-218.80
303.15	113.08	8.82	229.47	6.00	89.94	-219.80
308.15	113.20	7.98	229.66	6.15	90.18	-220.60
313.15	113.27	8.91	229.87	6.24	90.45	-221.65
318.15	113.34	8.96	230.03	6.29	90.65	-222.40
323.15	113.43	8.96	230.21	6.29	90.88	-223.15

Table 3.3(b): Partial Molal Volume, V_ϕ^0 ($cm^3\ mol^{-1}$) of aqueous Sugars and Urea solution at different temperature.

Temp. K	D-Glucose		Maltose		Urea	
	ϕ_v^0	S_v	ϕ_v^0	S_v	ϕ_v^0	S_v
293.15	112.00	9.02	227.94	6.00	91.86	-140.85
298.15	112.03	9.31	228.08	5.28	91.92	-139.65
303.15	112.07	9.75	228.12	5.71	92.06	-138.95
308.15	112.11	10.54	228.18	6.10	92.30	-140.45
313.15	112.20	10.35	228.25	6.38	92.44	-139.85
318.15	112.28	10.59	228.32	6.67	92.52	-138.80
323.15	112.36	10.79	228.45	6.04	92.63	-137.85

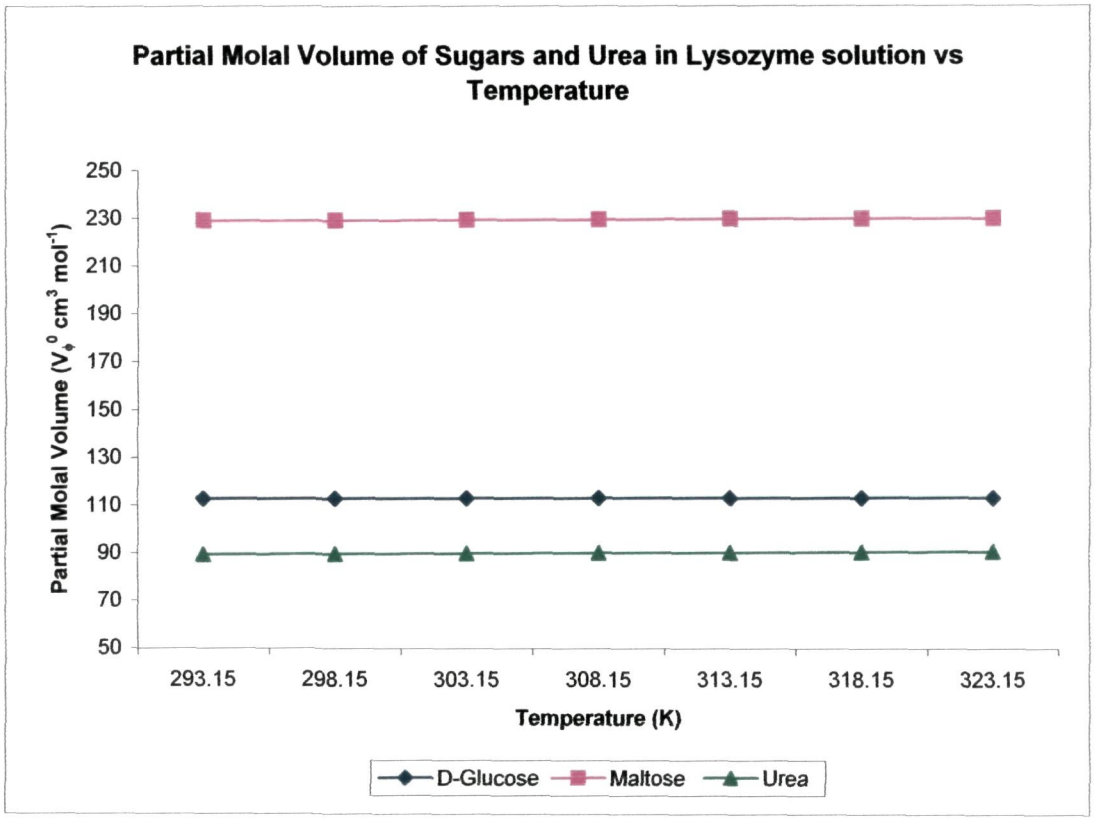
Partial Molal Volume of Sugars and Urea in Lysozyme solution vs Temperature
Partial Molal Volume (V_ϕ^0 cm^3 mol^{-1})
250
230
210
190
170
150
130
110
90
70
50
293.15
298.15
303.15
308.15
313.15
318.15
323.15
Temperature (K)
D-Glucose
Maltose
Urea

Fig. 3.3

Table 3.4: Transfer Volumes, $V^0_{\phi tr}$ ($cm^3\,mol^{-1}$) of Sugar and Urea from aqueous Lysozyme solution to water solution at different Temperatures.

Temp. K	D-Glucose	Maltose	Urea
293.15	0.88	1.09	-2.44
298.15	0.95	1.17	-2.24
303.15	1.01	1.35	-2.12
308.15	1.09	1.48	-2.12
313.15	1.07	1.62	-1.99
318.15	1.06	1.71	-1.87
323.15	1.07	1.76	-1.75

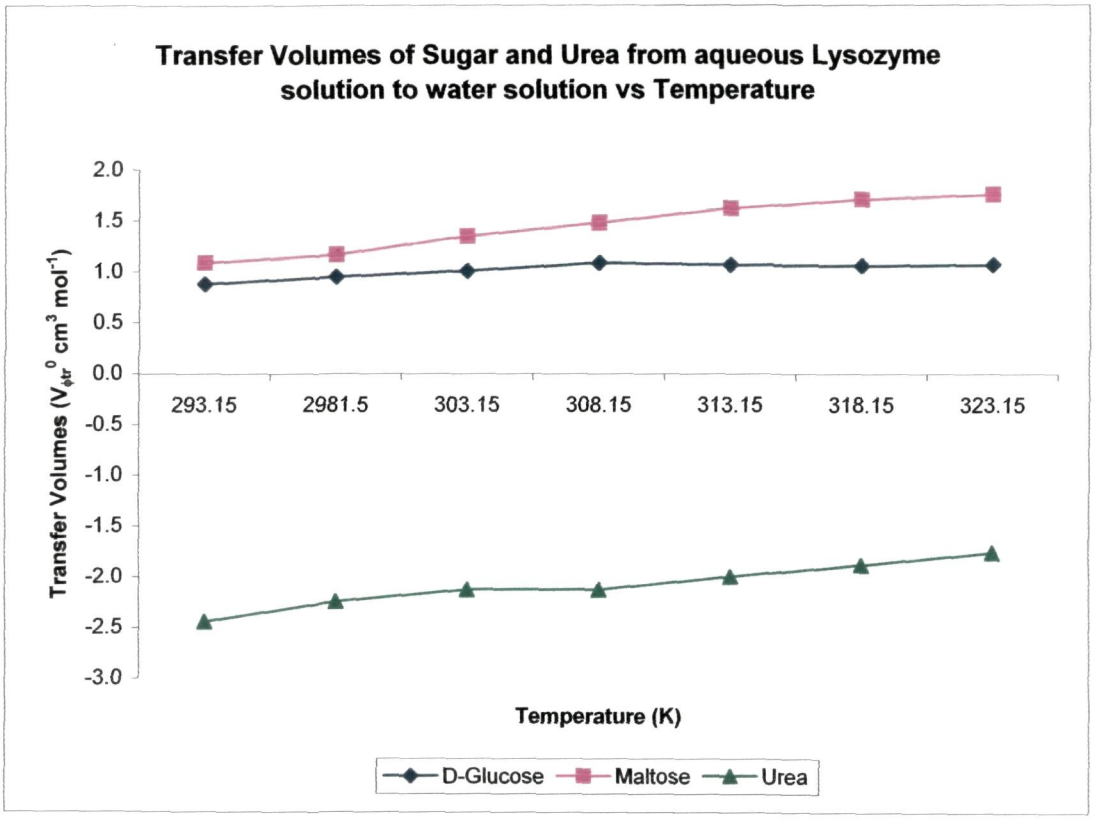
Transfer Volumes of Sugar and Urea from aqueous Lysozyme solution to water solution vs Temperature
Transfer Volumes ($V_{\phi tr}^{0}$ cm^3 mol^{-1})
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
293.15
2981.5
303.15
308.15
313.15
318.15
323.15
Temperature (K)
D-Glucose
Maltose
Urea

Fig. 3.4

hydrophilic R group of lysozyme present on the exterior are responsible for the negative values of transfer volumes [Table 3.4].

Ultrasonic velocity (U) measurements have been performed using a variable path ultrasonic interferometer at a frequency of 2 MHz. The ultrasonic velocities of lysozyme + sugar or urea + water system are summarized in [Tables 3.5(a-c)]. Adiabatic compressibility has been computed from ultrasonic velocity and density data using the equation,

$$\beta_s = 1/u^2\rho.$$

The adiabatic compressibility, β_s obtained from the above equation decreases with increases in temperature and composition as shown by Figs. 3.6(a-c) and Tables 3.6(a-c). The decrease in compressibility with increase in the thermal breaking of the solvent components, which in turn results in greater attractive forces among the molecules of a solution. Decrease in the β_s values with increase in composition is due to greater attractive forces among the molecules of a liquid.

Compressibility lowering ($\Delta\beta$) [45] was evaluated as the difference in the compressibility coefficients,

$$\Delta\beta_s = \beta_s^0 - \beta_s$$

in which β_s and β_s^0 are the compressibilities of solution and solvent, respectively. Figs. 3.7(a-c) represent the variation of compressibility lowering with the molal concentration of glucose, maltose and urea. There is a linear relationship between compressibility lowering and solute concentration. The compressibility lowering increases as the concentration of glucose

Table 3.5(a): Ultrasonic Velocities, U (ms^{-1}) of D(-) Glucose in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0610	0.0810	0.1010
293.15	1486.04	1486.08	1487.70	1489.30	1490.76	1492.28
298.15	1500.04	1501.70	1502.00	1502.50	1504.00	1505.56
303.15	1511.24	1511.30	1512.00	1513.52	1514.52	1516.16
308.15	1522.32	1522.50	1522.84	1523.20	1524.00	1528.50
313.15	1532.00	1531.40	1533.00	1533.80	1534.88	1536.40
318.15	1536.28	1540.04	1541.12	1542.20	1542.92	1543.32
323.15	1542.72	1543.60	1544.56	1545.80	1546.28	1550.60

Table 3.5(b): Ultrasonic Velocities, U (ms^{-1}) of Maltose in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0610	0.0820	0.1030
293.15	1486.04	1487.68	1488.56	1489.00	1491.08	1493.36
298.15	1500.04	1501.00	1501.58	1502.32	1503.92	1505.88
303.15	1511.24	1513.00	1514.00	1513.88	1516.64	1519.08
308.15	1522.32	1522.82	1523.12	1523.80	1527.20	1529.28
313.15	1532.00	1532.65	1533.00	1533.84	1537.28	1538.28
318.15	1536.28	1541.50	1542.52	1543.76	1544.44	1545.40
323.15	1542.72	1544.60	1545.88	1546.40	1549.96	1550.92

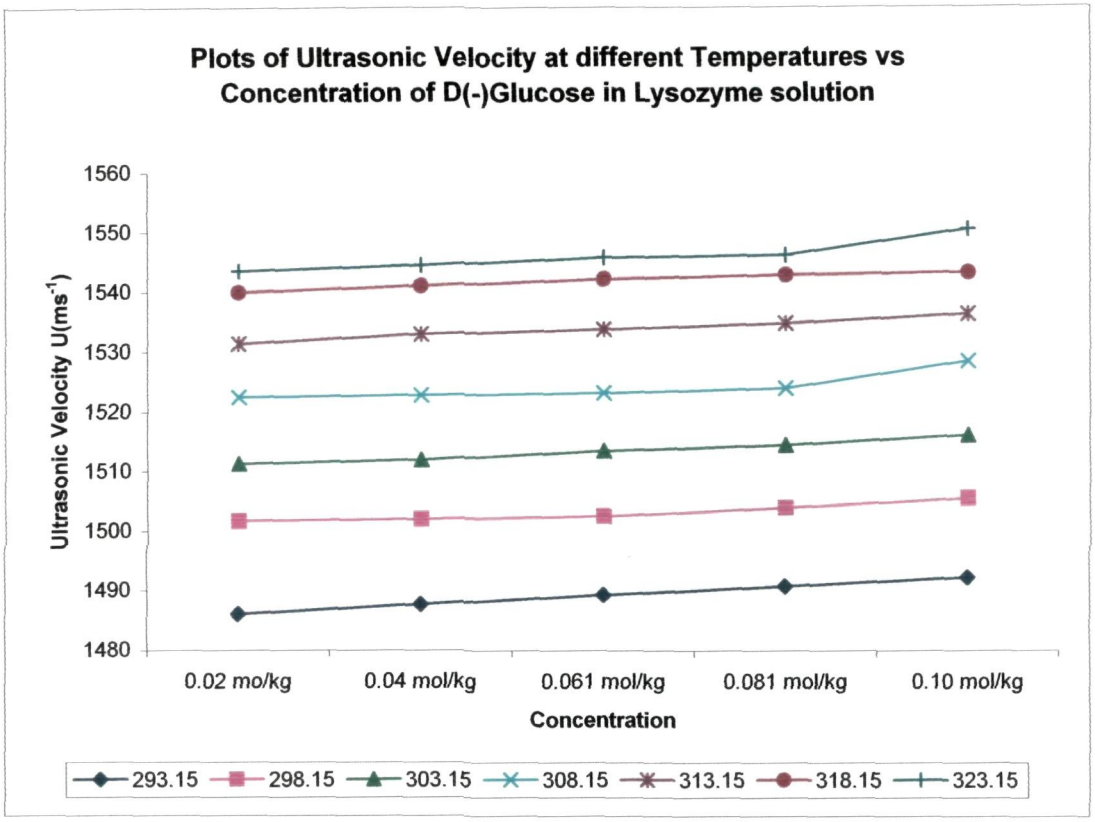

Fig. 3.5(a)

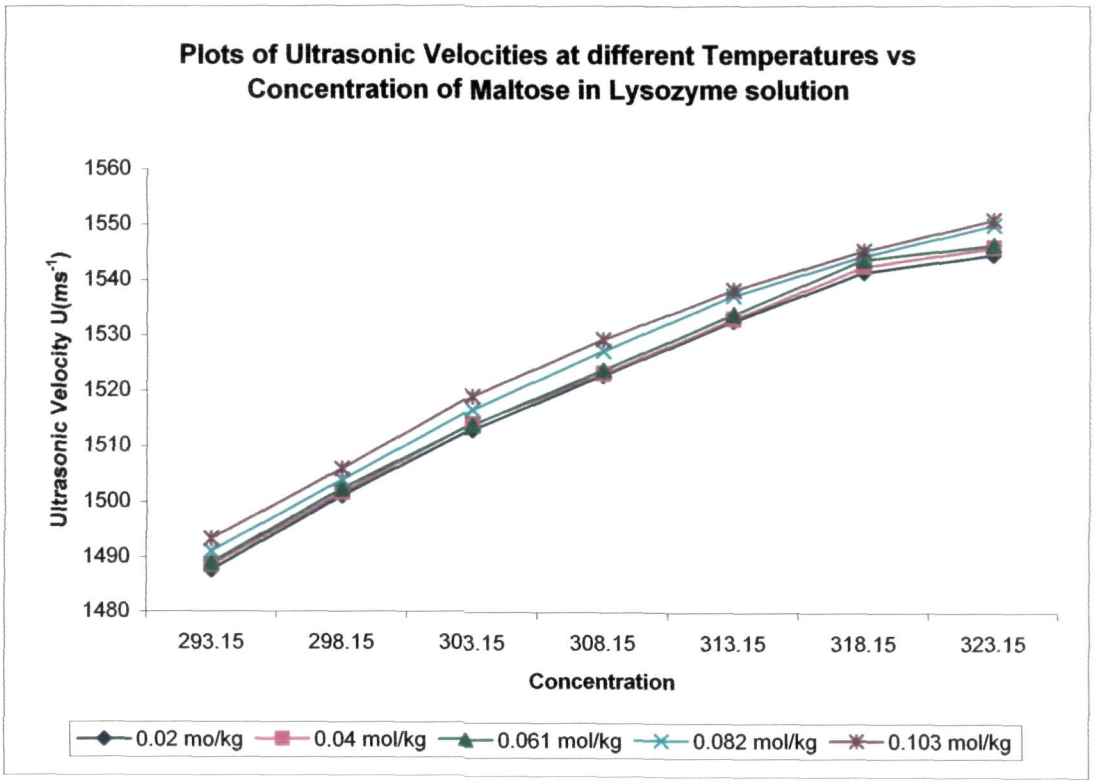

Fig. 3.5(b)

Table 3.5(c): **Ultrasonic Velocities, U (ms^{-1}) of Urea in aqueous Lysozyme solution as Functions of Concentration and Temperature.**

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0600	0.0800	0.1000
293.15	1486.04	1484.16	1483.72	1484.48	1485.44	1486.40
298.15	1500.04	1497.20	1497.60	1497.92	1498.82	1499.72
303.15	1511.24	1510.56	1509.36	1509.76	1510.30	1510.84
308.15	1522.32	1520.60	1521.80	1520.12	1520.52	1520.92
313.15	1532.00	1529.88	1528.92	1528.48	1529.70	1530.92
318.15	1536.28	1537.28	1537.20	1536.08	1538.14	1540.18
323.15	1542.72	1542.68	1542.88	1541.92	1546.08	1550.24

Table 3.6(a): **Adiabatic Compressibility ($\beta_s \times 10^{-7}$ cm^2 $dyne^{-1}$) of D(-) Glucose in aqueous Lysozyme solution as Functions of Concentration and Temperature.**

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0610	0.0810	0.1010
293.15	4.5243	4.5179	4.5022	4.4863	4.4718	4.4569
298.15	4.4500	4.4341	4.4265	4.4174	4.4029	4.3882
303.15	4.3940	4.3877	4.3778	4.3630	4.3516	4.3366
308.15	4.3389	4.3320	4.3243	4.3163	4.3062	4.2753
313.15	4.2983	4.2912	4.2765	4.2662	4.2546	4.2407
318.15	4.2583	4.2504	4.2388	4.2270	4.2175	4.2099
323.15	4.2493	4.2387	4.2278	4.2151	4.2070	4.1782

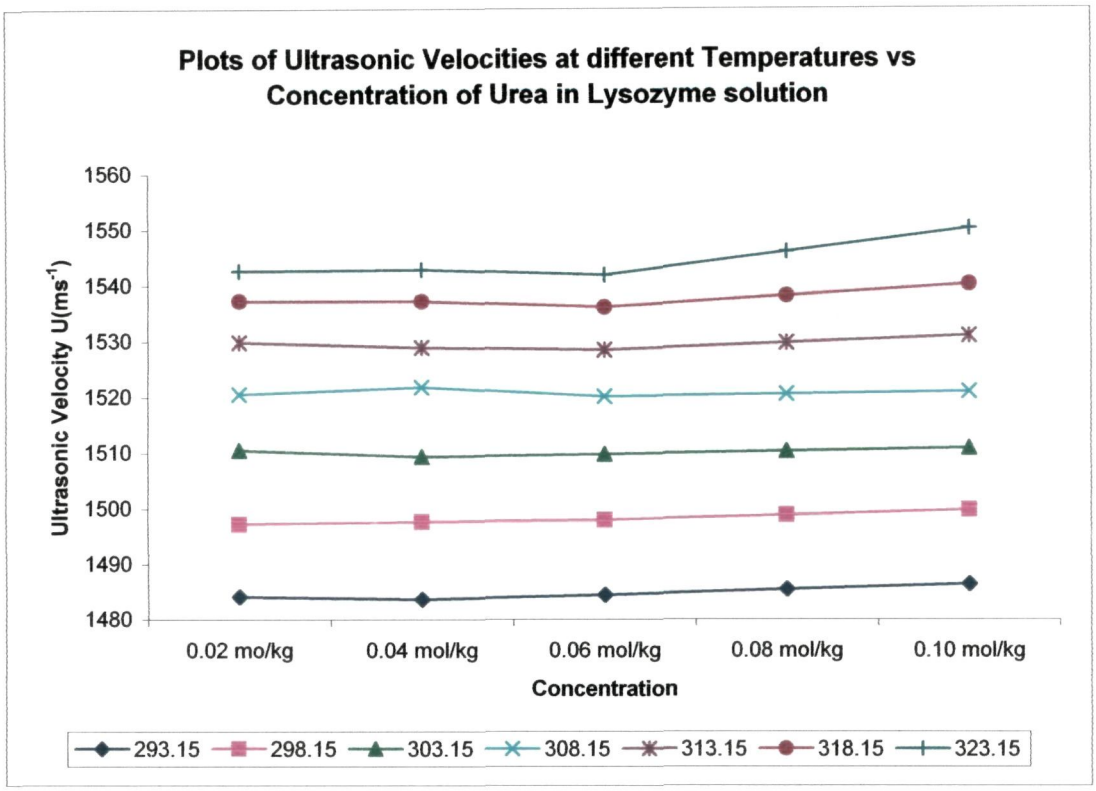

Fig. 3.5(c)

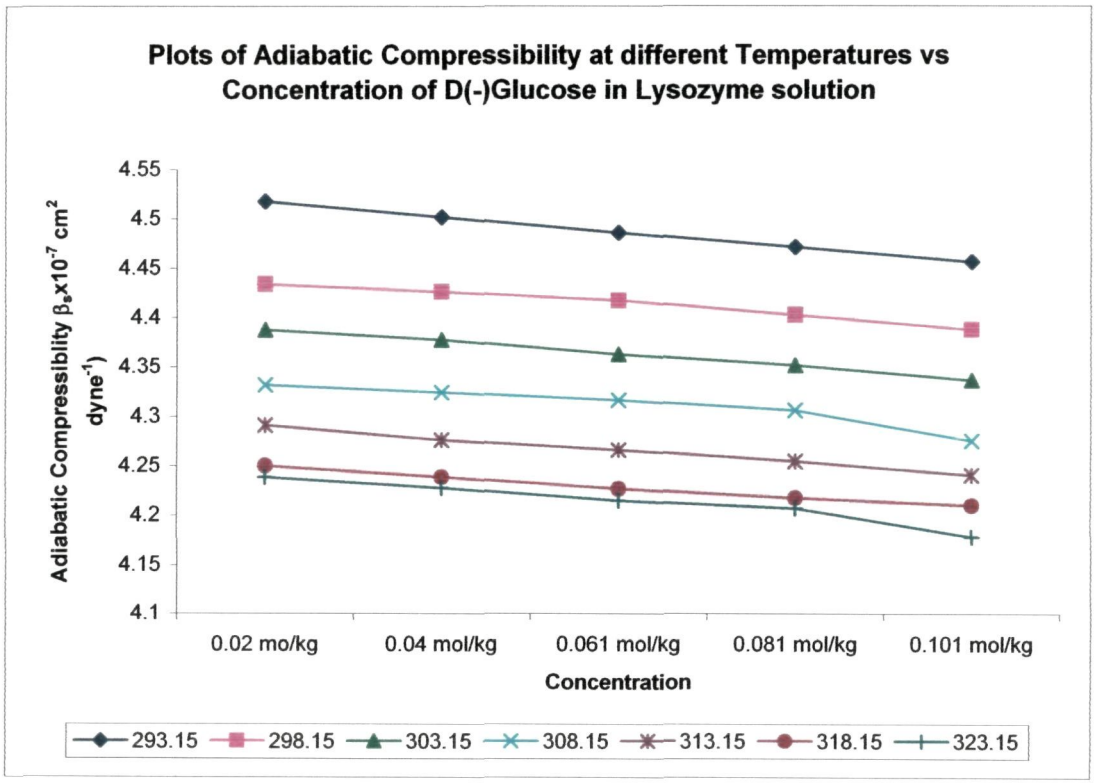

Fig. 3.6(a)

Table 3.6(b): **Adiabatic Compressibility ($\beta_s \times 10^{-7}$ cm^2 $dyne^{-1}$) of Maltose in aqueous Lysozyme solution as Functions of Concentration and Temperatures.**

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0610	0.0820	0.1030
293.15	4.5243	4.5025	4.4857	4.4711	4.4470	4.4219
298.15	4.4500	4.4326	4.4178	4.4017	4.3808	4.3581
303.15	4.3940	4.3722	4.3552	4.3443	4.3171	4.2921
308.15	4.3389	4.3247	4.3118	4.2964	4.2660	4.2434
313.15	4.2983	4.2787	4.2657	4.2496	4.2194	4.2030
318.15	4.2583	4.2369	4.2204	4.2023	4.1875	4.1713
323.15	4.2493	4.2278	4.2098	4.1957	4.1653	4.1493

Table 3.6(c): **Adiabatic Compressibility ($\beta_s \times 10^{-7}$ cm^2 $dyne^{-1}$) of Urea in aqueous Lysozyme solution as Functions of Concentration and Temperature.**

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0610	0.0820	0.1030
293.15	4.5243	4.5379	4.5425	4.5378	4.5318	4.5259
298.15	4.4500	4.4689	4.4684	4.4664	4.4610	4.4556
303.15	4.3940	4.4000	4.4089	4.4065	4.4032	4.4000
308.15	4.3389	4.3508	4.3458	4.3553	4.3529	4.3506
313.15	4.2983	4.3076	4.3149	4.3173	4.3103	4.3034
318.15	4.2583	4.2735	4.2758	4.2820	4.2704	4.2591
323.15	4.2493	4.2516	4.2523	4.2575	4.2345	4.2118

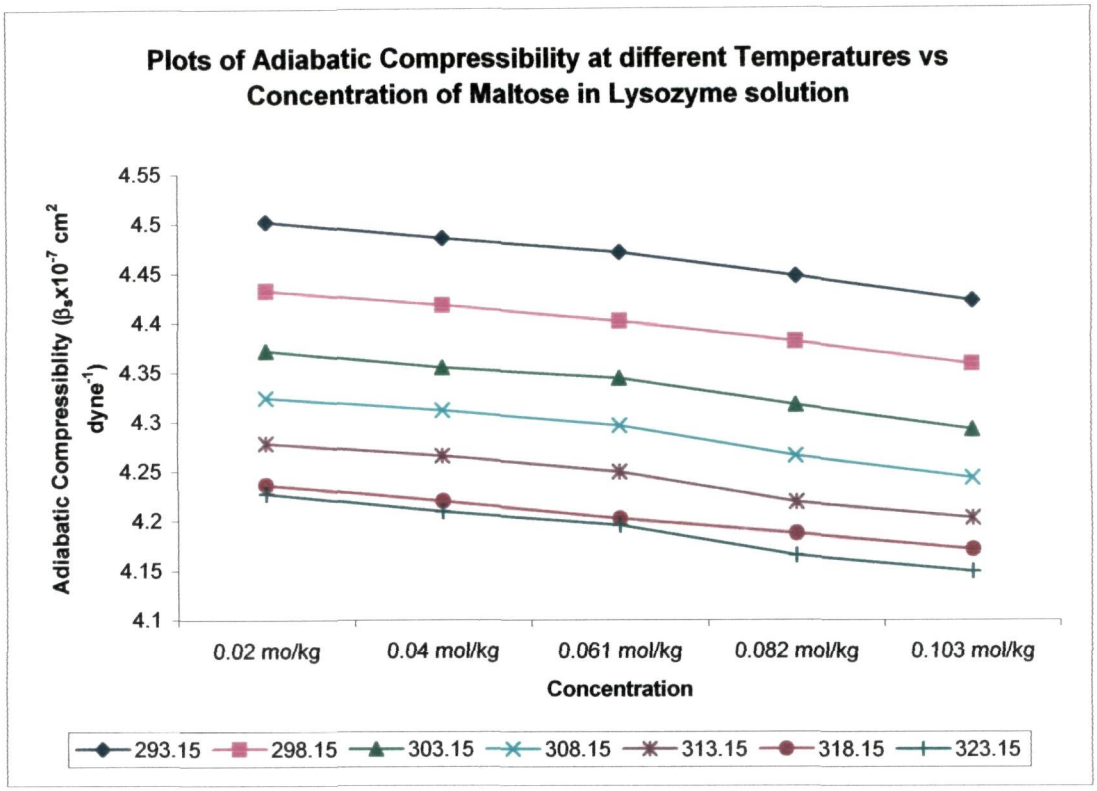

Fig. 3.6(b)

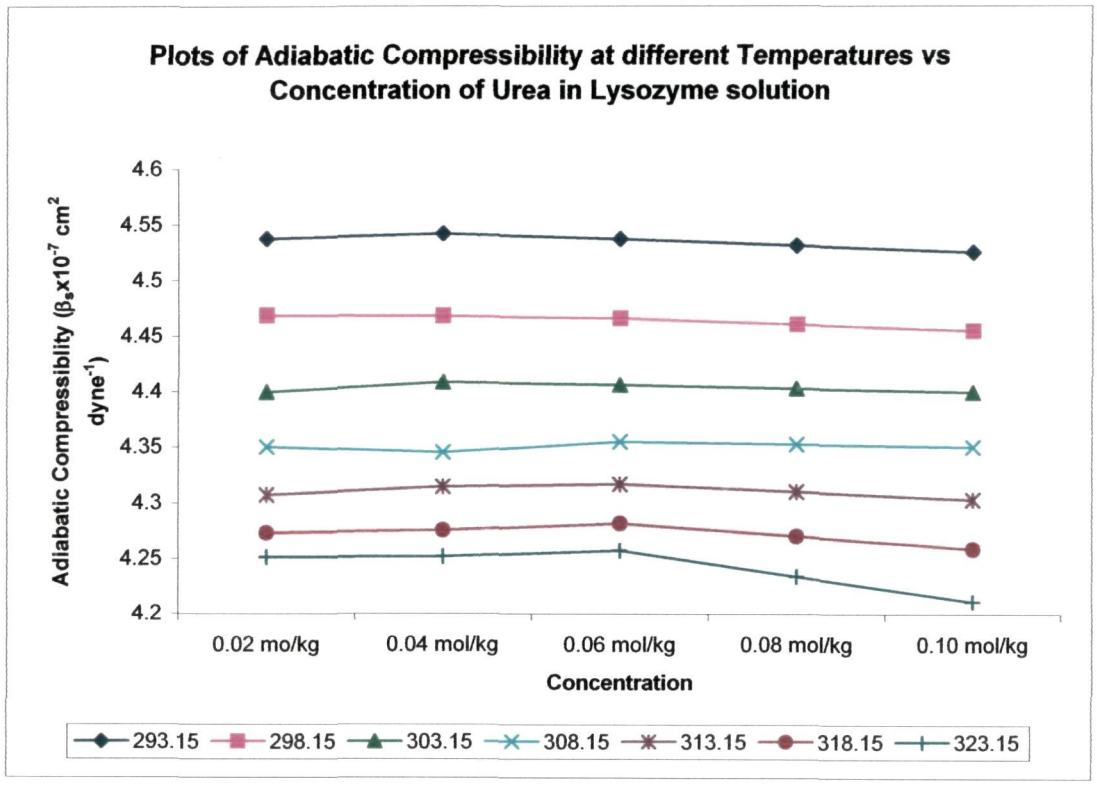

Fig. 3.6(c)

Table 3.7(a): **Compressibility Lowering ($\Delta\beta_s \times 10^{-7}$ cm^2 dyne^{-1}) of D(-) Glucose in aqueous Lysozyme solution as Functions of Concentration and Temperature.**

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0810	0.1010
293.15	0.0043	0.0182	0.0319	0.0443	0.0570
298.15	0.0044	0.0196	0.0265	0.0389	0.0515
303.15	0.0044	0.0124	0.0251	0.0344	0.0473
308.15	0.0049	0.0108	0.0167	0.0247	0.0335
313.15	0.0053	0.0134	0.0217	0.0312	0.0430
318.15	0.0058	0.0156	0.03254	0.0328	0.0383
323.15	0.0080	0.0178	0.0284	0.0345	0.0410

Table 3.7(b): **Compressibility Lowering ($\Delta\beta_s \times 10^{-7}$ cm^2 dyne^{-1}) of Maltose in aqueous Lysozyme solution as Functions of Concentration and Temperature.**

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0820	0.1030
293.15	0.0204	0.036	0.0489	0.0715	0.0947
298.15	0.0159	0.0295	0.0439	0.0633	0.0843
303.15	0.0204	0.0362	0.0455	0.0712	0.0945
308.15	0.0128	0.0245	0.0382	0.0672	0.0881
313.15	0.0136	0.0254	0.0399	0.0686	0.0834
318.15	0.0198	0.0352	0.0417	0.0651	0.0795
323.15	0.0202	0.0370	0.0495	0.0784	0.0927

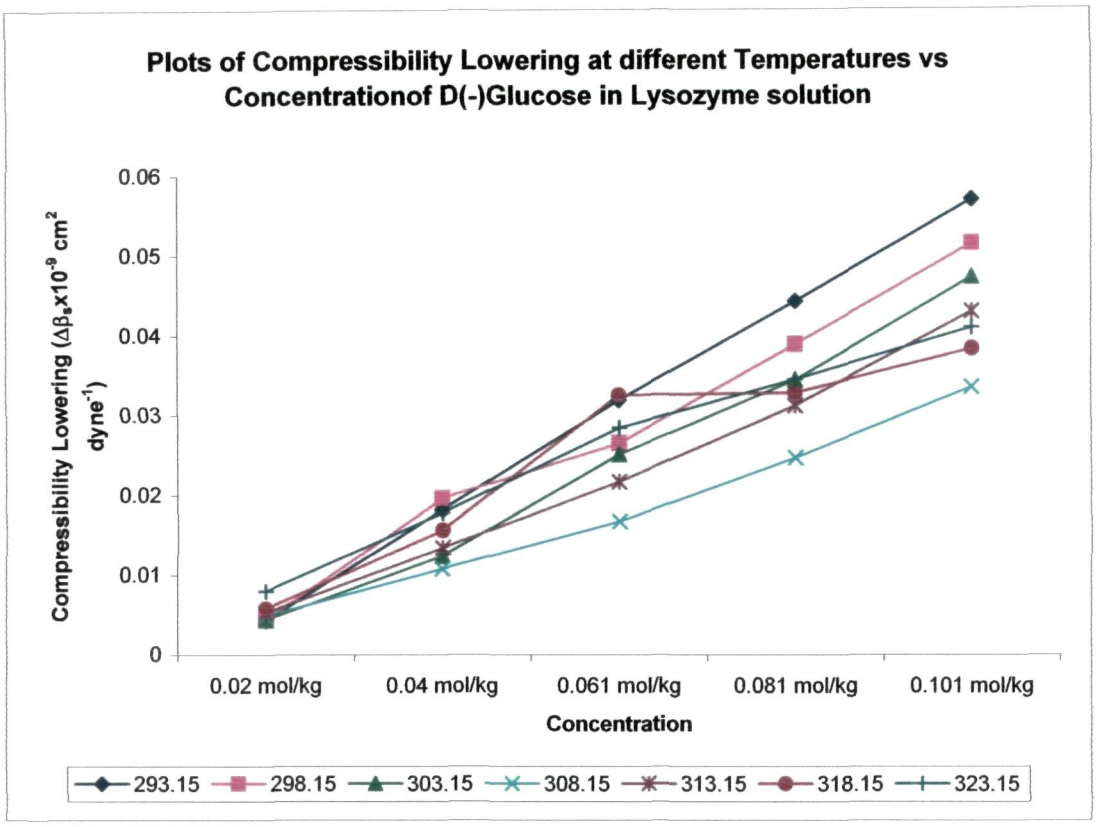

Fig. 3.7(a)

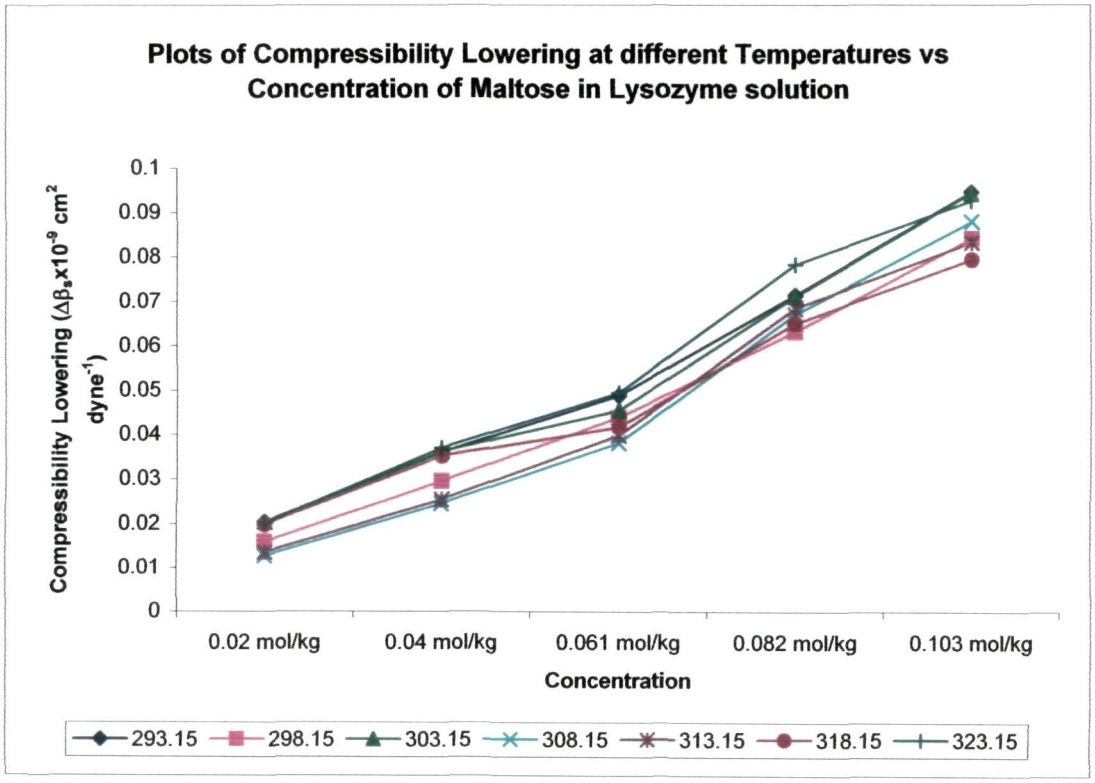

Fig. 3.7(b)

Table 3.7(c): Compressibility Lowering ($\Delta\beta_s \times 10^{-7}$ cm^2 dyne^{-1}) of Urea in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0600	0.0800	0.1000
293.15	-0.0137	-0.0178	-0.0122	-0.0063	-0.0000
298.15	-0.0191	-0.0181	-0.0157	-0.0099	-0.0041
303.15	-0.0061	-0.0144	-0.0117	-0.0129	-0.0045
308.15	-0.0120	-0.0065	-0.0157	-0.0155	-0.0102
313.15	-0.0141	-0.0208	-0.0228	-0.0112	-0.0082
318.15	-0.0155	-0.0172	-0.0231	-0.0159	-0.0060
323.15	-0.0024	-0.0026	-0.0074	-0.0784	-0.0100

Table 3.8(a): Apparent Molal Adiabatic Compressibility ($K_\phi \times 10^{-6}$ cm^3 mol^{-1} dyne^{-1}) of D(-) Glucose in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0810	0.1010
293.15	19.0663	-8.6203	-11.3507	-13.9633	-15.9887
298.15	-29.4661	9.0555	-3.3590	-8.1666	-11.3182
303.15	18.0447	12.4290	-1.4183	-3.0110	-7.6244
308.15	14.3740	5.0465	18.3570	8.4491	-14.5688
313.15	35.5444	-.9753	2.9676	0.2706	-4.5821
318.15	8.3421	-6.2126	-3.6459	-2.7371	-0.2929
323.15	-5.4821	-5.6313	-8.6496	-4.7878	-23.4381

Table 3.8(b): Apparent Molal Adiabatic Compressibility ($K_\phi \times 10^{-6}$ cm^3 mol^{-1} $dyne^{-1}$) of Maltose in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0820	0.1030
293.15	-5.7342	6.4400	15.43662	7.8852	2.2160
298.15	14.5496	20.7885	21.7926	16.1465	10.8370
303.15	-9.0113	2.7101	18.0908	5.1677	-0.5258
308.15	27.9732	31.0143	28.7796	8.7941	4.4878
313.15	22.3162	27.3772	24.8327	5.7737	8.0430
318.15	-10.5021	1.5530	4.1626	9.3851	10.9557
323.15	11.3421	-2.8381	7.8935	-7.4923	-2.3981

Table 3.8(c): Apparent Molal Adiabatic Compressibility ($K_\phi \times 10^{-6}$ cm^3 mol^{-1} $dyne^{-1}$) of Urea in aqueous Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0600	0.0800	0.1000
293.15	106.2840	83.0657	56.4578	41.5070	32.6683
298.15	132.4900	83.1551	60.8993	32.8986	36.2695
303.15	67.4980	74.0872	54.0762	42.9280	36.3767
308.15	96.8983	53.6189	60.3472	48.6973	41.8470
313.15	106.3390	89.2742	72.1314	51.6589	39.5040
318.15	113.3130	80.0306	72.3345	45.9169	30.3871
323.15	48.1258	43.3403	46.1694	11.7475	-8.6119

or maltose increases and in the case of lysozyme + urea + water system, the compressibility lowering generally decreases as the concentration of urea increases.

Sugar molecules interact with water thus increasing the order of water molecules in the solvation layer, which gives rise to unfavourable decrease in entropy of water. The non-polar groups of protein side chains decreases the extent of solvation because each group no longer presents its entire surface to the solution. This results in increase in entropy of protein solution, which is the major deriving force for association of hydrophobic groups in aqueous solution, thereby causing the cluster of amino acid side chains in the protein interior. Thus, the hydrophobic groups are more exposed in the unfolded state, partially exposed in molten globule and not exposed towards solvent molecules in natured state as is reflected by a decrease in compressibility and compressibility lowering values [Tables 3.6(a-c) and 3.7(a-c)].

The apparent molal adiabatic compressibility (K_ϕ) of sugars or urea in aqueous lysozyme solutions at different temperatures ranging from 293.15 to 323.15 K, summarized in Tables 3.7(a-c) was calculated from the following equation,

$$K_\phi = \frac{1000\,(\beta_s \rho_0 - \beta_s^{\,0} \rho)}{m \rho \rho_0} + \frac{\beta_s M}{\rho}.$$

It has been observed that after the addition of different sugars to the lysozyme solution there is an increase in the values of apparent molal volumes and decrease in compressibility of the solutions. This may be attributed to the fact that the addition of sugars to the protein increases the hydrophobic, electrostatic, and hydrogen-bonding

interaction giving rise to the compact form of protein. Therefore, by observing a decrease in the compressibility of the solution and the increase in the apparent molal volume of the protein after the addition of sugars, we can say that the extent of denaturation of protein is reduced and its stabilization has taken place.

No definite trend is observed in K_ϕ values with temperature. Most of the K_ϕ values are negative when glucose and maltose are solutes while the values of K_ϕ are found to be positive when urea is the solute. The negative values of K_ϕ [Tables 3.8(a-c)] indicate electrostriction and hydrophobic interactions. The values of K_ϕ for sugar + aqueous lysozyme system are found to be negative at all temperatures, which can be explained by postulating that polar OH groups of sugars interact with surrounding solvent water through dipole-dipole interaction in such a way that the surrounding water loses its own compressibility to a certain extent, and degree of organization of water molecules increases by forming clusters in the vicinity of protein, so it will limit the denaturation of protein. The unfavourable (or polar) environment produced by sugar molecules will increase the hydrophobic interaction in proteins thus increasing its stabilization.

Urea may be regarded as alkaline in water, acting as a proton donor and acceptor and hence in a mixture of urea and water, the structure is likely to be broken [46]. Frank's theory [47] suggests that urea plays the role of a statistical structure breaker.

Proteins are stabilized by a combination of hydrogen-bonding, electrostatic and hydrophilic interactions. In some proteins, there is an additional contribution from cross-linking,

metal complexing and specific binding of ions and cofactors. In discussing the effect of different sugars on the stability of lysozyme, we have to consider the effect of different sugars on these various forces and the interactions.

In aqueous solutions of protein, there is a cooperative hydrogen bonded structure [48] in which water competes as donor and acceptor with the backbone and side chain groups of the protein. When sugar is added to the protein solution, the OH groups of sugars may also compete for hydrogen binding [48]. Now we have to consider the respective interactions between protein, water and additive (sugar) molecules. The additive interacting more strongly with protein than with water will tend to stabilize the denatured state by the formation of protein sugar complexes. They will, therefore, have a denaturing effect. However, additives interacting more strongly with water molecules than with protein will favour the stabilization of protein molecules [49]. In the present case, the sugar interacts more strongly with water molecules than with protein by forming hydrogen bonds with water molecules. This will favour an increase in the degree of organisation of water molecules by the formation of clusters (as in ice) and will thus limit the unfolding of protein [49]. This was supported by the view that the addition of sugars results in a higher resistance of proteins to denaturation by an increased contribution of water extrusion entropy change [50].

The aqueous solutions of sugars have lower dielectric constant than pure water indicating that the electrostatic interactions are stronger in these solutions than in pure water as reported in the literature [51]. The above mentioned interactions between sugar and

water molecules create a polar environment near the protein due to which the hydrophobic interactions increase. These hydrophobic interactions are generally considered to be the significant factor in stabilizing the three dimensional structure of proteins [48,52]. In aqueous-organic mixed solvents, hydrophobic interactions depend on the solvent structure, with maximum hydrophobic interactions occurring in those solvent mixtures in which the three-dimensional structure of water is most developed or the degree of water molecules organization is increased [49,53]. The effect of sugars on hydrophobic interactions and consequently on the thermal stability of proteins should also depend upon how they affect the structure of water. Hydrophobic interactions between pairs of hydrophobic groups are stronger in sugar solutions than in pure water [54]. It seems likely therefore that this is the mechanism by which sugars in general may stabilize proteins to heat denaturation. Evidence derived from both spectroscopy and thermodynamic studies shows that sugars interact with water to an extent, which depends upon their molecular structure [55, 56]. Glucose behaves differently from sucrose, for example, and mannitol behaves differently from sorbitol [57]. Taiet et al. (1972) have proposed a "specific hydration model" to explain these effects. Sugar molecules induce structure in the water molecules, surrounding them if the orientation of OH groups is such that some of the O-O spacing corresponds with the O-O distance of 4.86 Å of the water lattice.

These results strongly support the hypothesis that the dominant mechanism by which sugars stabilize proteins to heat denaturations is through their effect on the structure of water, which in turn, determines the strength of hydrophobic interactions.

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Chapter-4

Effect of Glucose, Maltose and Urea on the stabilization of Lysozyme solution in terms of viscosity, its related parameters and thermodynamic properties

The study of viscous behaviour of macromolecules in solution is important in understandings the mechanism of transport processes.

In proteins, processes involving conformational changes are shown by medium viscosity [1]. The effect of viscosity on the rate of protein-dependent chemical reaction was originally described by Kramers [2]. Kramers' treatment was applied to protein folding and to other protein processes involving structural movements, such as folding or catalysis [3,4]. Following synthesis, proteins acquire a number of different conformations before reaching the "native" form. Likewise, denaturing involves passage through different unfolded states [5]. In enzymes, during catalysis or ligand binding, conformational changes occur, at least in the active site [6]. Furthermore, many enzymes may exhibit widely different structural conformations, distinguishable by protease sensitivity, antibody recognition, circular dichroism or fluorescence [7]. Thus, according to Kramers' theory enzymes alternating between widely different conformations during catalysis should be inhibited by viscosity [1]. One such case is carbon-monoxy-myglobin embedded in a trehalose glass matrix, where trehalose inhibits the release of carbon mono oxide [8].

Hen egg white lysozyme is a well-known enzyme that acts as a glycoside hydrolase. This small globular protein consists of two functional domains located on each side of the active site cleft and contains both helices and regions of β sheet, together with loop regions, turns and disulfide bridges [9].

Very little attention has been paid to the viscosity of lysozyme aqueous solutions [10] and data of viscosity of lysozyme in mixed aqueous solutions are rare. Viscosity of egg-white lysozyme was measured in the presence of carbohydrate additives in reaction medium. These additives show a significant affinity for water. They depress water activity and increase the viscosity of the medium [11]. Solute-solvent interactions in aqueous solutions of the additives are characterized by properties such as the intrinsic viscosity and B-coefficient. In this work, viscosity measurements have been carried on sugar or urea + aqueous lysozyme solutions (keeping the concentration of aqueous lysozyme solution constant) at different temperatures for different concentrations of sugar or urea to understand the increased or decreased stability of lysozyme in presence of sugars or urea respectively.

The thermodynamic stability of the native structure of proteins has provided one of the great challenges in biochemistry and currently remains the subject of extensive investigation [12]. In an idealistic approach, the thermodynamic information would be obtained by measuring the changes associated with the unfolding of the protein, from its native state to a reference state. The latter should be independent of the initial state of the protein. Presumably, a suitable reference state would be one where the protein is extended in such a way that there remains minimal interaction between amino acids' side chains and each constituent amino acid is optimally accessible to the solvent. An obvious obstacle to this simple procedure, however, is that known means of inducing protein unfolding (temperature, addition of chemicals, change in pH etc.); usually do not lead to the required

well-defined final state [12-13]. In the case of chemical denaturation, further complication arises, since the comparison of initial and final states reflects thermodynamic changes due to the changes in solvent composition as well as from the protein structural modifications.

Heating of proteins in solution can lead to aggregation, gelation, denaturation, and thermal expansion, etc; depending upon the temperature range. The solute-solvent, solvent-solvent, and solute-solute interactions in a protein solution undergo substantial changes upon exposure to different temperatures that bring about the observable physical change in the protein solution. As the thermal environment is altered, the Gibbs free energy, ΔG^* of the system changes, altering the physical state of the protein for which ΔG^* is minimized.

Results and Discussion

Viscosities of sugars + aqueous lysozyme and urea + aqueous lysozyme systems are shown in Tables 4.1(a-c) for different molality of solute at different temperatures. The increase in concentration of solute increases the viscous behaviour of the solution due to an increase in number of solute molecules, which causes more frictional resistance to the flow. But if we see in the case of urea in lysozyme solution, viscosity first decreases from 0.02 mol/kg to 0.06 mol/kg and then it gradually increases. Therefore, we can say that at lower concentration of urea, its structure breaking effect is more pronounced while at higher concentrations, its effect is only to increase the viscosity of the solution like any other solute.

Table 4.1(a): Viscosity ($\eta \times 10^4$, kg $m^{-1}s^{-1}$) of D-Glucose in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0610	0.0810	0.1010
293.15	1.0139	1.0351	1.0456	1.0532	1.0622	1.0713
298.15	0.9010	0.9067	0.9125	0.9243	0.9331	0.9495
303.15	0.8071	0.8335	0.8421	0.8507	0.8563	0.8619
308.15	0.7287	0.7490	0.7664	0.7749	0.7834	0.7859
313.15	0.6619	0.6868	0.6937	0.7036	0.7091	0.7190
318.15	0.6051	0.6193	0.6290	0.6343	0.6441	0.6479
323.15	0.5560	0.5685	0.5736	0.5818	0.5914	0.6010

Table 4.1(b): Viscosity ($\eta \times 10^4$, kg $m^{-1}s^{-1}$) of Maltose in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0610	0.0820	0.1030
293.15	1.0139	1.0869	1.1035	1.1188	1.1248	1.1339
298.15	0.9010	0.9276	0.9451	0.9614	0.9731	0.9894
303.15	0.8071	0.8197	0.8308	0.8405	0.8563	0.8660
308.15	0.7287	0.7395	0.7639	0.7719	0.7815	0.8001
313.15	0.6619	0.6757	0.6924	0.7003	0.7218	0.7267
318.15	0.6051	0.6112	0.6336	0.6457	0.6594	0.6641
323.15	0.5560	0.5633	0.5780	0.5988	0.6122	0.6197

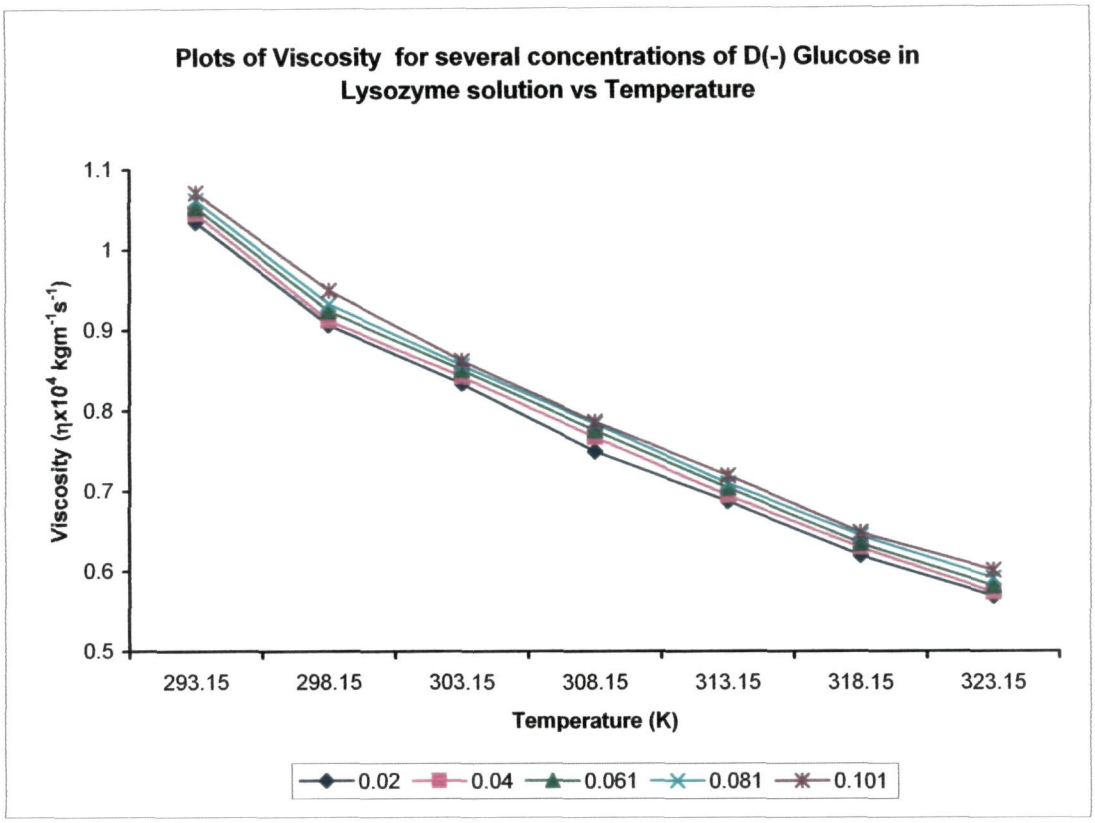

Fig. 4.1(a)

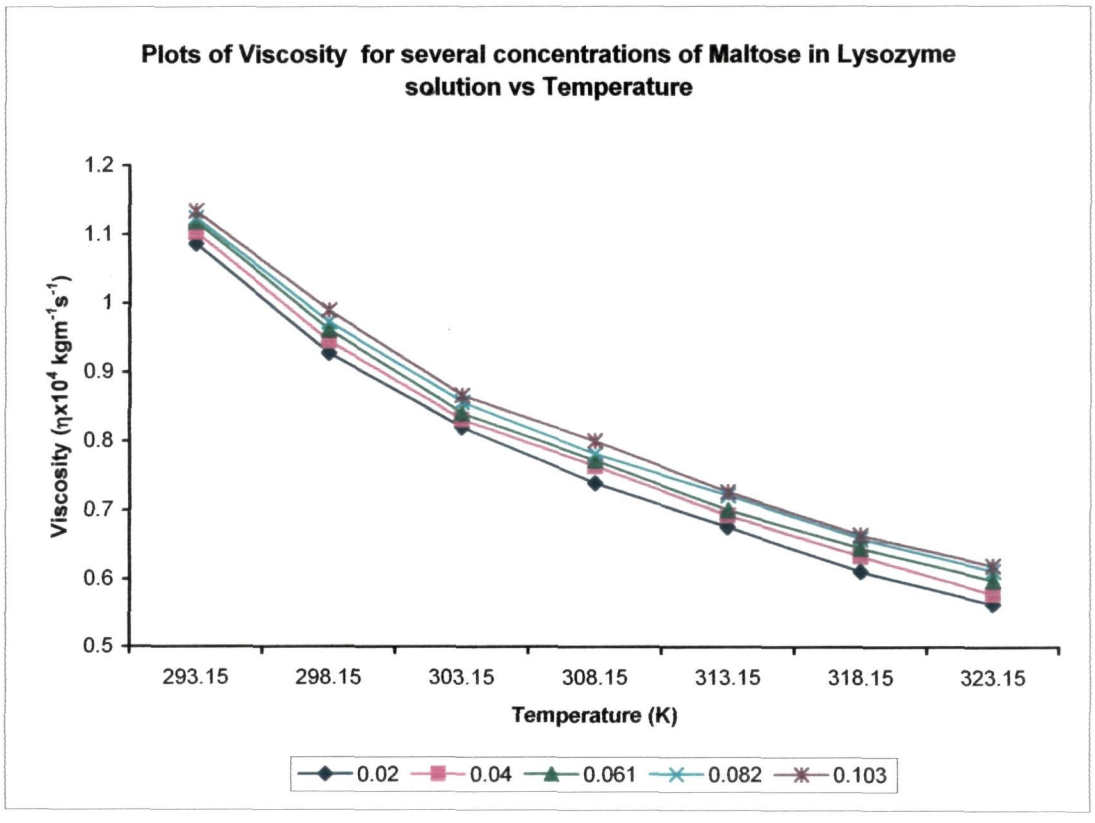

Fig. 4.1(b)

Table 4.1(c): Viscosity ($\eta \times 10^4$, kg $m^{-1}s^{-1}$) of Urea in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0000	0.0200	0.0400	0.0600	0.0800	0.1000
293.15	1.0139	1.0622	1.0450	1.0374	1.0420	1.0435
298.15	0.9010	0.9217	0.9047	0.8971	0.9017	0.9032
303.15	0.8071	0.8276	0.8019	0.7975	0.8019	0.8005
308.15	0.7287	0.7313	0.7221	0.7191	0.7265	0.7236
313.15	0.6619	0.6676	0.6524	0.6554	0.6628	0.6584
318.15	0.6051	0.6033	0.6001	0.5957	0.5972	0.6002
323.15	0.5560	0.5528	0.5467	0.5482	0.5482	0.5482

Table 4.2(a): Relative Viscosity, η_r of D-glucose in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0810	0.1010
293.15	1.0209	1.0313	1.0388	1.0477	1.0567
298.15	1.0064	1.0128	1.0259	1.0357	1.0539
303.15	1.0328	1.0434	1.0541	1.0610	1.0679
308.15	1.0279	1.0518	1.0635	1.0751	1.0786
313.15	1.0375	1.0480	1.0630	1.0712	1.0862
318.15	1.0234	1.0395	1.0483	1.0644	1.0707
323.15	1.0224	1.0317	1.0464	1.0636	1.0809

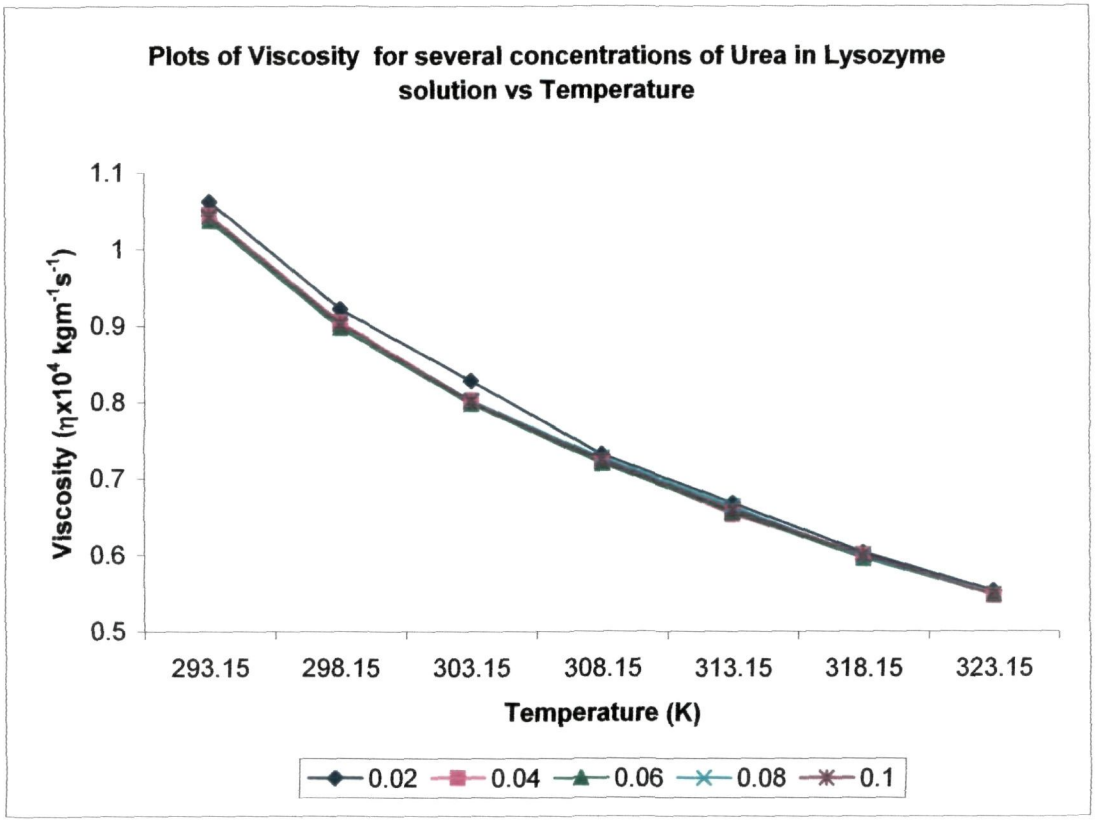

Fig. 4.1(c)

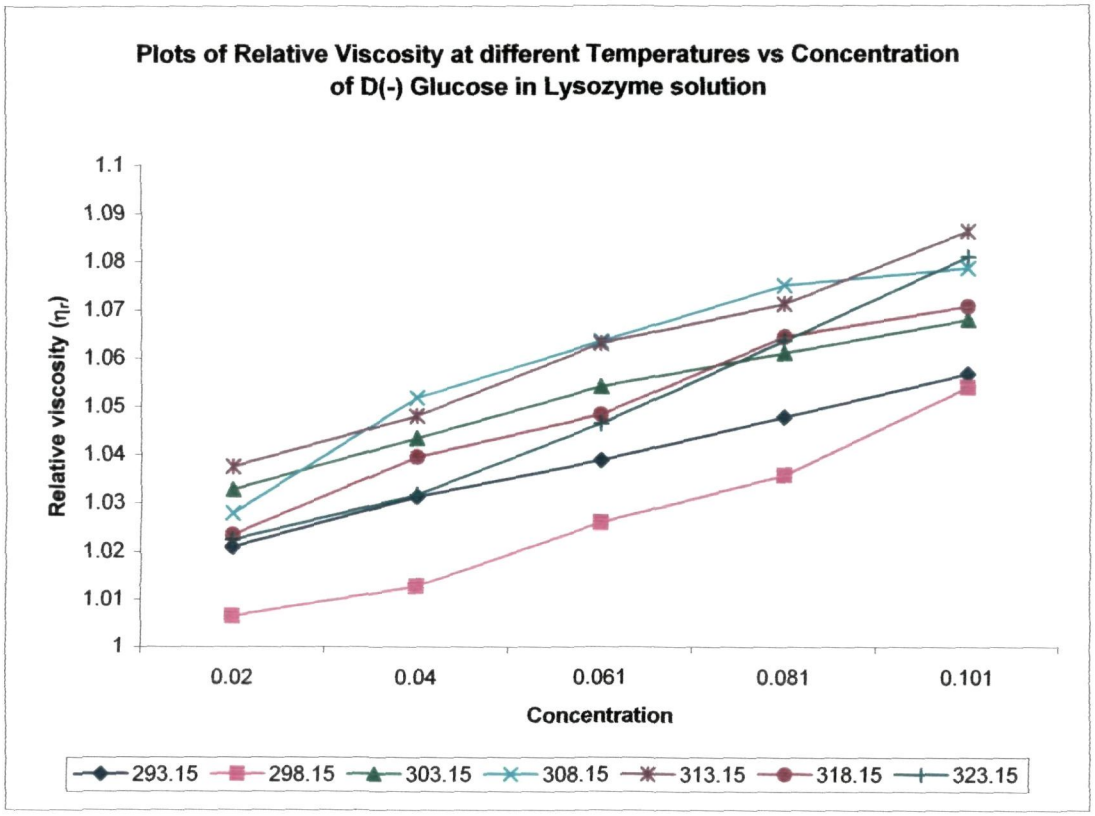

Fig. 4.2(a)

Table 4.2(b): Relative Viscosity, η_r of Maltose in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0820	0.1030
293.15	1.0721	1.0885	1.1035	1.1094	1.1184
298.15	1.0295	1.0491	1.0671	1.0801	1.0982
303.15	1.0156	1.0293	1.0414	1.0609	1.0730
308.15	1.0149	1.0483	1.0593	1.0725	1.0980
313.15	1.0207	1.0461	1.0580	1.0905	1.0979
318.15	1.0100	1.0470	1.0671	1.0897	1.0975
323.15	1.0132	1.0396	1.0769	1.1011	1.1146

Table 4.2(c): Relative Viscosity, η_r of Urea in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0600	0.0800	0.1000
293.15	1.0477	1.0307	1.02319	1.0277	1.0292
298.15	1.0230	1.0041	0.9958	1.0008	1.0025
303.15	1.0253	0.9936	0.9881	0.9936	0.9918
308.15	1.0036	0.9909	0.9869	0.9971	0.9930
313.15	1.0085	0.9856	0.9901	1.0014	0.9946
318.15	0.9971	0.9918	0.9844	0.9869	0.9918
323.15	0.9942	0.9833	0.9860	0.9860	0.9860

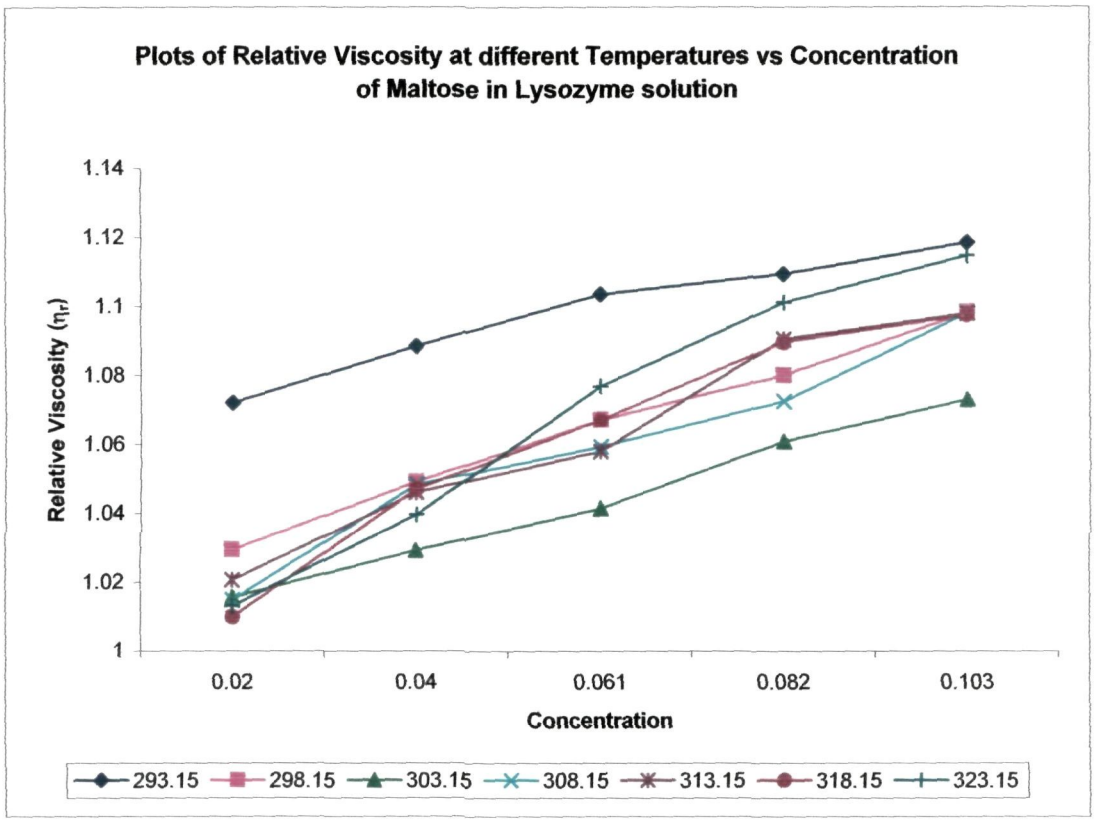

Fig. 4.2(b)

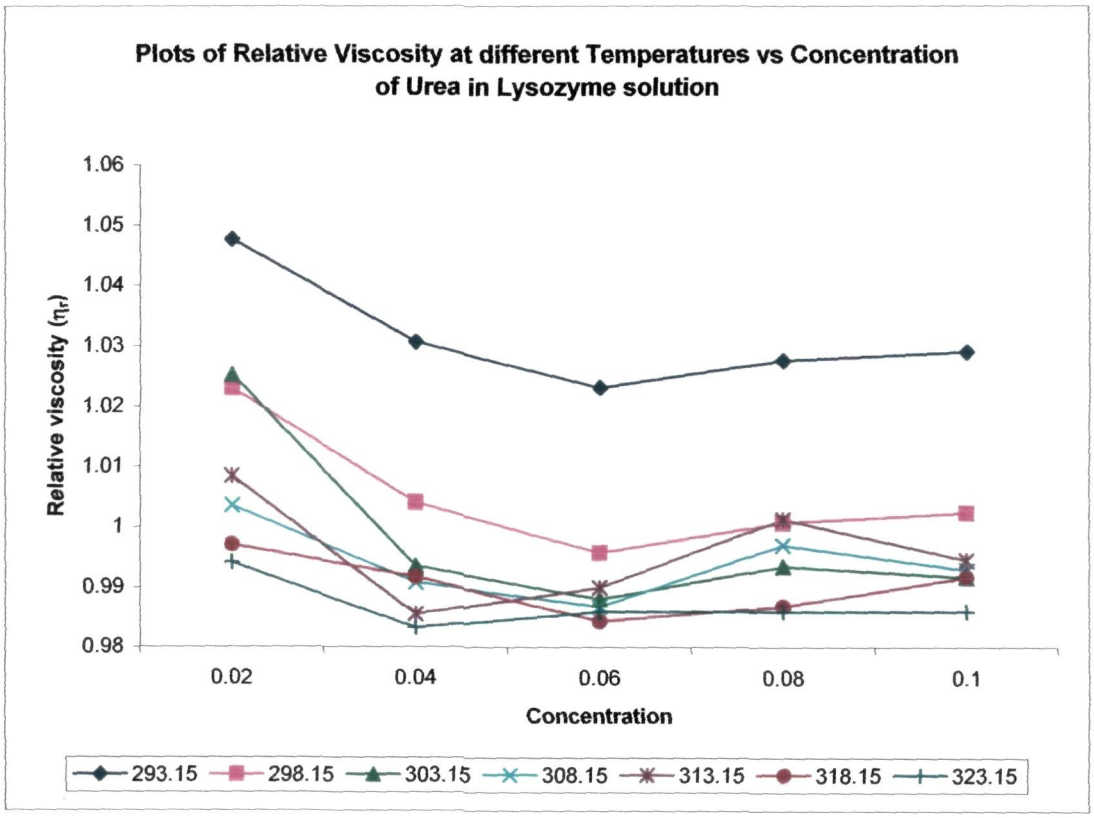

Fig. 4.2(c)

The increase in concentration of solute in solution contributes positively to the viscosity B-coefficient. On the other hand, the breaking of the solvent structure by solute causes a decrease in the viscosity. This contributes negatively to the B-coefficient. Thus, B-coefficient is the resultant of these two opposite forces [14]. Therefore, the urea molecules exhibiting negative B-coefficient have been assumed to exert a structure breaking effect on the solvent while the ions with positive B-coefficient exert a structure making effect on the solvent.

It has been observed [Table 4.3] that all the values of viscosity B-coefficient for saccharides are positive and in aqueous lysozyme solution, these values are greater for maltose than for glucose. B-coefficient depends directly on size, shape and charge of the solute molecules, and maltose has two glucose units joined by α-1,4-glucosidic linkage. Therefore, there is the order B(glucose) < B(maltose). It is noteworthy that the B(maltose) is not twice as large as that of B(D-glucose), indicating that the formation of α-1,4-linkage reduces the structure making effects of saccharides.

In aqueous solution of proteins, there is a cooperative hydrogen bonded structure [15], in which water competes as both donor and acceptor with backbone and side chain groups in the protein. When sugar is added to the protein solution, the individual OH groups of sugar may also compete for hydrogen bonding, but this effect is very small. The aqueous solutions of sugars have low dielectric constant [16] than pure water indicating that the electrostatic interactions should be stronger in

Table 4.3: B-coefficient ($dm^3\ mol^{-1}$) of sugars or urea in Lysozyme as Functions of Concentration and Temperature.

Temp. K	D-Glucose	Maltose	Urea
293.15	0.4400	0.5675	-0.2000
298.15	0.5895	0.8420	-0.2215
303.15	0.4390	0.7320	-0.3350
308.15	0.6235	0.9525	-0.0750
313.15	0.6030	0.9940	-0.0600
318.15	0.5975	1.0885	-0.0775
323.15	0.7445	1.3215	-0.0685

Table 4.4: The values of the Intrinsic Viscosity ([η], m^3/kg) and Huggins Coefficient, K for Sugars or Urea in Lysozyme solutions at different Temperatures.

Temp. K	[η]	K_1
D-Glucose		
293.15	1.0702	-4.9863
298.15	0.2421	-48.1383
303.15	1.6870	-3.9433
308.15	1.5669	-3.2059
313.15	1.8762	-3.3181
318.15	1.2275	-3.6784
323.15	1.0438	-2.8429
Maltose		
293.15	3.7249	-2.0494
298.15	1.5245	-2.6079
303.15	0.7602	-0.6186
308.15	1.9948	-3.1805
313.15	1.0929	-0.5516
318.15	0.7090	8.9152
323.15	0.6946	12.9107
Urea		
293.15	2.2177	-4.6838
298.15	0.9463	-13.0795
303.15	0.9331	-15.0113
308.15	0.0182	-4661.2730
313.15	0.2129	-64.0355
318.15	-0.2213	17.0704
323.15	-0.4139	15.8336

these solutions than in pure water. However, this contribution to the stabilizing effect must be relatively small as compared to the hydrophobic interactions.

Hydrophobic interactions are generally considered to be the significant factor in stabilizing the three-dimensional structure of proteins [15,17]. In aqueous-organic mixed solvents, hydrophobic interactions depend on the solvent structure, with maximum hydrophobic interactions occurring in those solvent mixtures in which the three-dimensional structure of water is most developed or the degree of water molecules organization is increased [18,19]. Tait et al. [20] have derived the evidence for the same fact from both spectroscopy and thermodynamics. The protective action of sugars on proteins can be attributed to the fact that sugars may replace a certain number of water molecules that are hydrogen-bonded to the structure in a way similar to water itself creating a hydrophilic surface. This would result in a solvent system where the already exposed side chains attached with non-polar groups in the native protein molecules would have a tendency to enter into the interior of protein due to the polar environment produced by sugar molecules. Similar groups in the interior of the protein would find even more unfavorable environment in sugar solutions than in pure water on their exposure. This phenomenon would be responsible for higher stability of the protein molecules in these solvents and would reduce the extent of denaturation of protein molecules induced thermally.

According to Feakin's model [21], greater the value of ΔG^*, the greater is the structure making ability of solute. A perusal of

Tables 4.7(a-c) shows that ΔG^* increases with increase in temperature. This, thereby, indicates that the structure making ability of solute increases.

Negative value of entropy of activation (ΔS^*) suggests that the attainment of transition state for viscous flow is accompanied by bond formation and increase in order.

The compact structure of protein has greater amount of hydrogen bonding and less exposed hydrophobic groups. The increased order of the water molecules in the solvation layer correlates with an unfavourable decrease in the entropy of water. The cluster of non-polar groups decreases the extent of solvation because each group no longer presents its entire surface to the solution. The result is a favourable increase in entropy of protein solution. The entropy is the major deriving force for the association of hydrophobic groups in aqueous solution. Hydrophobic amino acid side chains, therefore, tend to be clustered in the protein's interior away from water [22], thus giving rise to compact form of protein.

Table 4.5(a): $RT\ln(\eta v_m/hN)$ ($kJmol^{-1}$) of D-Glucose in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0810	0.1010
0.29315	57.0905	58.2979	59.1402	59.7486	60.2414
0.29815	57.7414	58.9601	59.8306	60.4518	60.9751
0.30315	58.5032	59.7521	60.6304	61.2547	61.7592
0.30815	59.1993	60.5014	61.3964	62.0421	62.5464
0.31315	59.9399	61.2293	62.1470	62.7950	63.3353
0.31815	60.6278	61.9524	62.8695	63.5478	64.0757
0.32315	61.3556	62.6833	63.6304	64.3222	64.8857

Table 4.5(b): $RT\ln(\eta v_m/hN)$ ($kJmol^{-1}$) of Maltose in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0820	0.1030
0.29315	58.3924	59.8155	60.7599	61.4314	61.9666
0.29815	59.0009	60.4570	61.4257	62.1254	62.6910
0.30315	59.6787	61.1516	62.1227	62.8506	63.4121
0.30815	60.4100	61.9502	62.9342	63.6581	64.2602
0.31315	61.1600	62.7052	63.7076	64.4897	65.0580
0.31815	61.8766	63.4761	64.5146	65.2847	65.8629
0.32315	62.6348	64.2319	65.3308	66.1161	66.7170

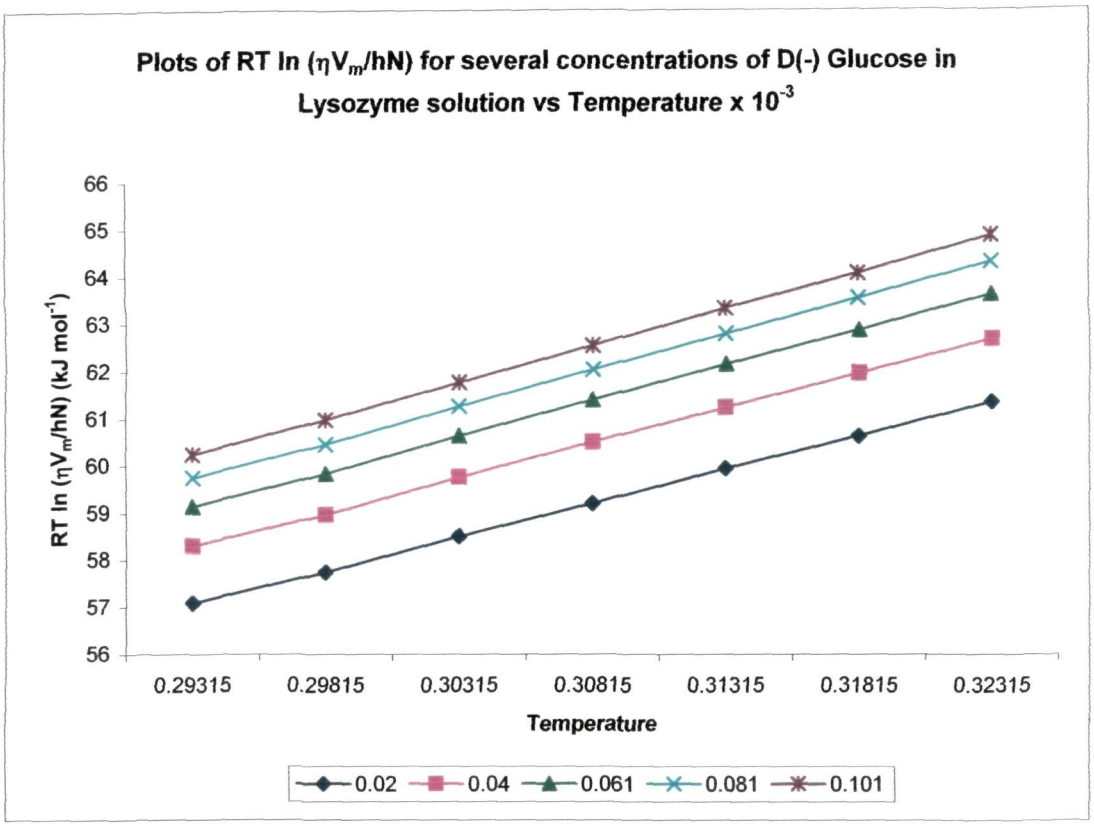

Fig. 4.3(a)

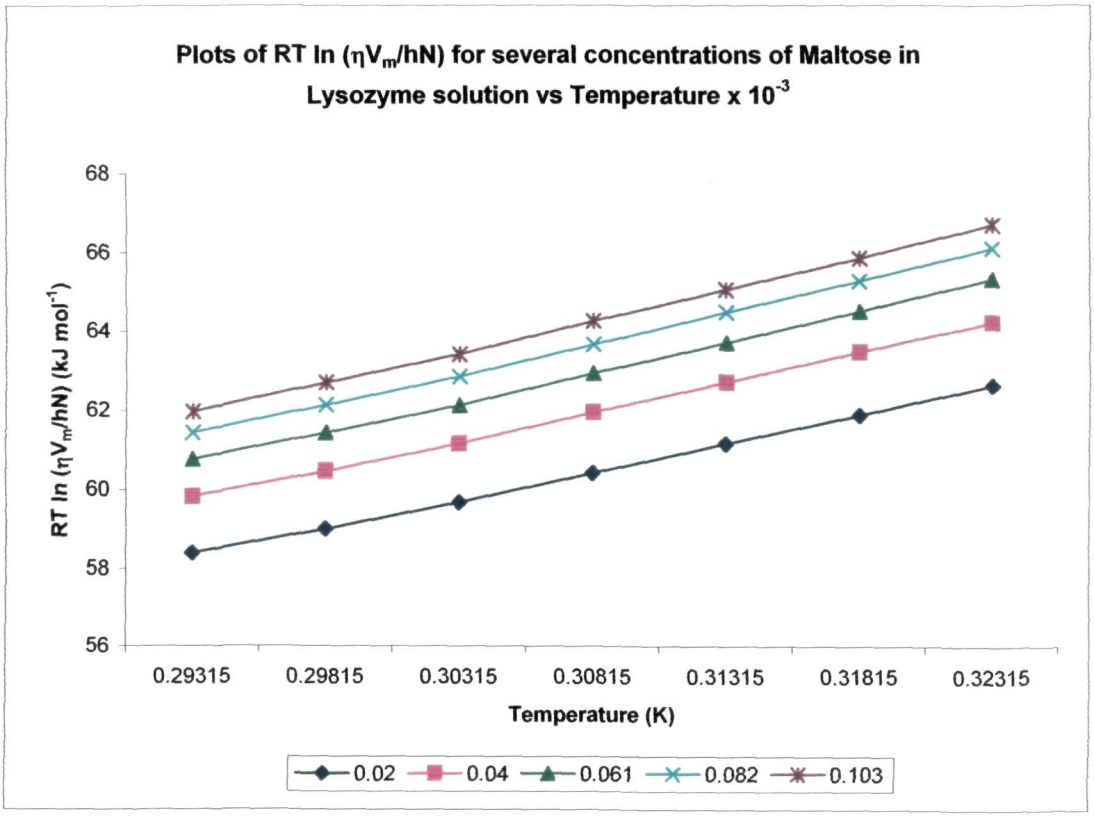

Fig. 4.3(b)

Table 4.5(c): $RT\ln(\eta v_m/hN)$ ($kJmol^{-1}$) of Urea in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0600	0.0800	0.1000
0.29315	55.8396	56.5482	57.1016	57.5747	57.9667
0.29815	56.4457	57.1607	57.7207	58.2037	58.6029
0.30315	57.1265	57.8209	58.3977	58.8897	59.2870
0.30815	57.7569	58.5112	59.1009	59.6131	60.0113
0.31315	58.4625	59.2020	59.8241	60.3472	60.7449
0.31815	59.1325	59.9307	60.5311	61.0396	61.4745
0.32315	59.8320	60.6272	61.2642	61.7738	62.2021

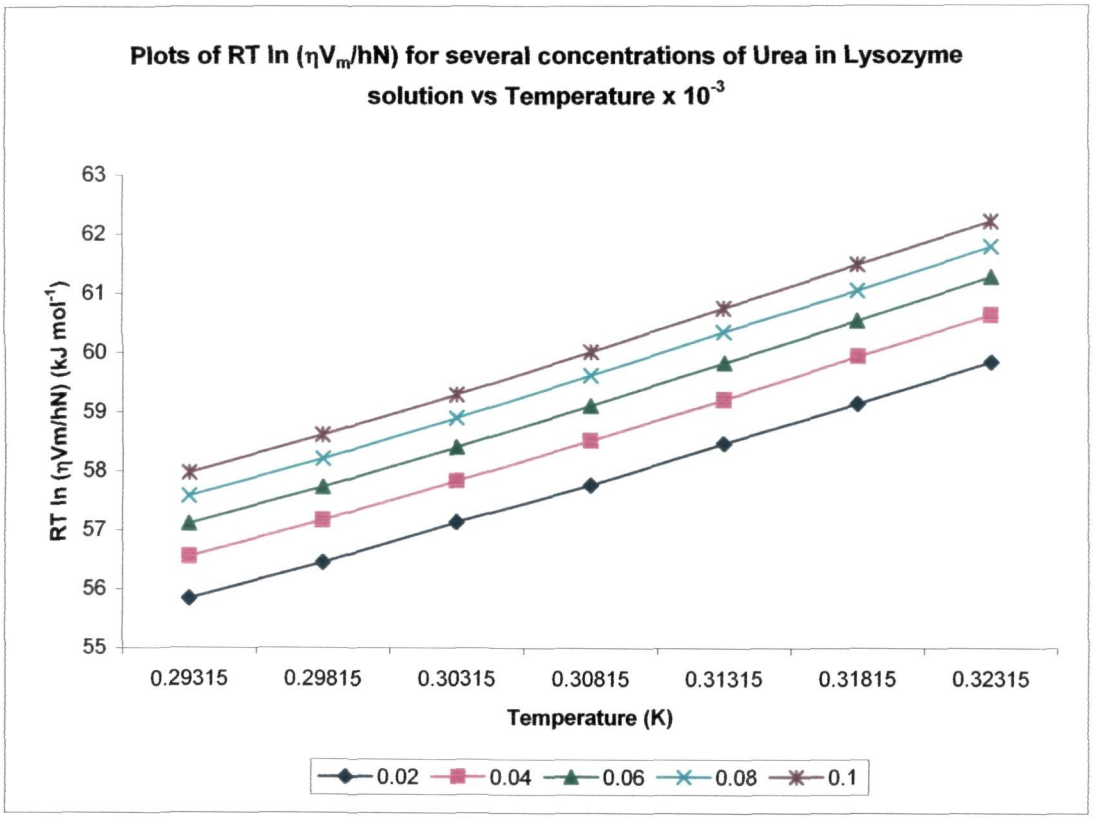
Plots of RT ln (ηV_m/hN) for several concentrations of Urea in Lysozyme solution vs Temperature x 10^{-3}
RT ln (ηVm/hN) (kJ mol^{-1})
63
62
61
60
59
58
57
56
55
0.29315
0.29815
0.30315
0.30815
0.31315
0.31815
0.32315
Temperature (K)
0.02
0.04
0.06
0.08
0.1

Fig. 4.3(c)

Table 4.6: Entropy (ΔS^*, kJ mol^{-1}) and Enthalpy (ΔH^*, kJ mol^{-1}) of sugars and urea in Lysozyme solution as a Function of Concentration.

Molality (mol kg^{-1})	ΔS^* kJ mol^{-1}	ΔH^*, kJ mol^{-1}
D(-) Glucose		
0.0200	-142.8914	15.1763
0.0400	-147.2714	15.1007
0.0610	-150.4643	15.0122
0.0810	-153.2364	14.8034
0.1010	-155.0729	14.7998
Maltose		
0.0200	-142.5779	16.5153
0.0400	-148.8643	16.0971
0.0610	-153.3957	15.7019
0.0820	-157.2271	15.2585
0.1030	-158.8636	15.3273
Urea		
0.0200	-133.4771	16.6684
0.0400	-136.8436	16.3746
0.0600	-139.5357	16.1364
0.0800	-140.9043	16.2149
0.1000	-142.1950	16.2240

Table 4.7(a): Free Energy of Activation for viscous flow, ΔG^* (kJ mol^{-1}) of D(-) Glucose in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0610	0.0810	0.1010
293.15	57.0649	58.2733	59.1208	59.7247	60.2594
298.15	57.7794	59.0097	59.8731	60.4908	61.0348
303.15	58.4938	59.7460	60.6255	61.2570	61.8102
308.15	59.2083	60.4824	61.3778	62.0232	62.5855
313.15	59.9227	61.2187	62.1301	62.7894	63.3609
318.15	60.6372	61.9551	62.8824	63.5556	64.1362
323.15	61.3517	62.6915	63.6347	64.3217	64.9116

Table 4.7(b): Free Energy of Activation for viscous flow, ΔG^* (kJ mol^{-1}) of Maltose in lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.040	0.0610	0.0820	0.1030
293.15	58.3120	59.7367	60.6698	61.3496	61.8982
298.15	59.0249	60.4810	61.4368	62.1358	62.6925
303.15	59.7378	61.2253	62.2038	62.9219	63.4868
308.15	60.4507	61.9696	62.9708	63.7080	64.2811
313.15	61.1636	62.7140	63.7378	64.4942	65.0754
318.15	61.8765	63.4583	64.5047	65.2803	65.8698
323.15	62.5893	64.2026	65.2717	66.0664	66.6641

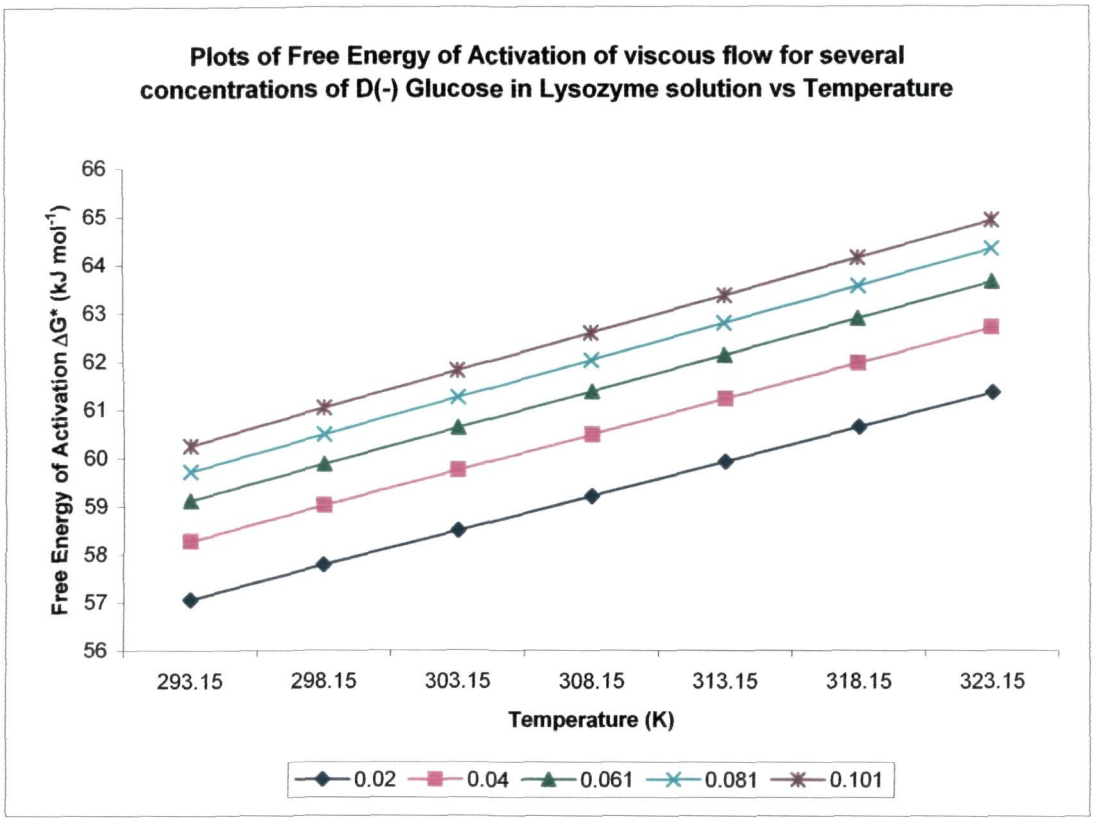

Fig. 4.4(a)

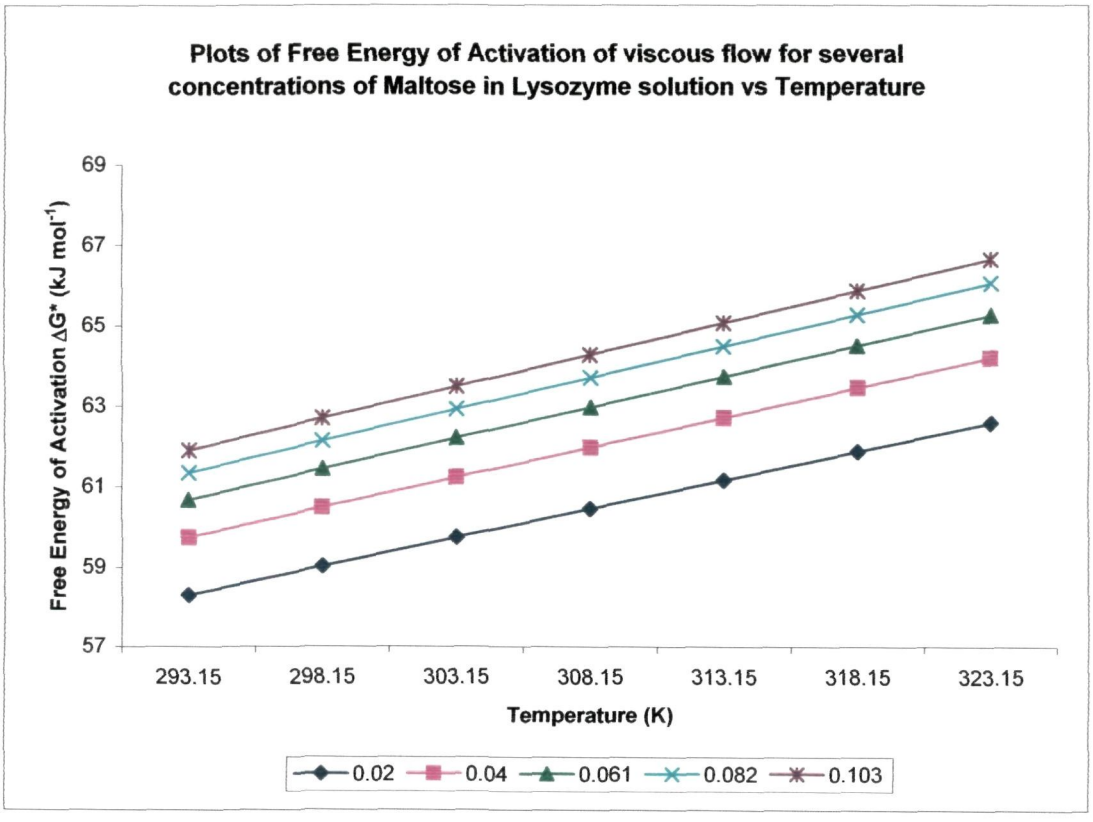

Fig. 4.4(b)

Table 4.7(c): Free Energy of Activation for viscous flow, ΔG^* (kJ mol^{-1}) of Urea in Lysozyme solution as Functions of Concentration and Temperature.

Molality mol kg^{-1} / Temp. K	0.0200	0.0400	0.0600	0.0800	0.1000
293.15	55.7972	56.4903	57.0413	57.5210	57.9084
298.15	56.4646	57.1745	57.7390	58.2255	58.6194
303.15	57.1320	57.8587	58.4367	58.9300	59.3304
308.15	57.7994	58.5430	59.1343	59.6346	60.0414
313.15	58.4668	59.2272	59.8320	60.3391	60.7524
318.15	59.1341	59.9114	60.5297	61.0436	61.4633
323.15	59.8015	60.5956	61.2274	61.7481	62.1743

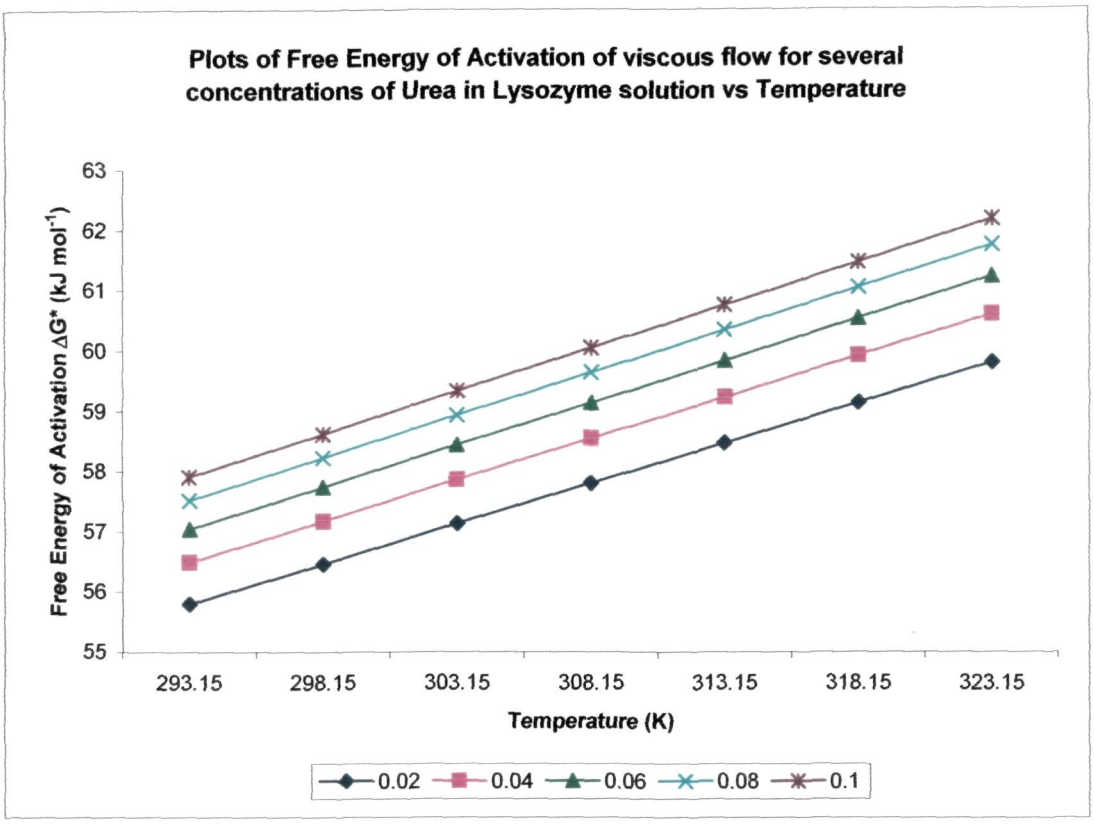
Plots of Free Energy of Activation of viscous flow for several concentrations of Urea in Lysozyme solution vs Temperature
Free Energy of Activation ΔG* (kJ mol-1)
63
62
61
60
59
58
57
56
55
293.15
298.15
303.15
308.15
313.15
318.15
323.15
Temperature (K)
0.02
0.04
0.06
0.08
0.1

Fig. 4.4(c)

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